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FLUCTUATIONS IN AGE COMPOSITION AND GROWTH RATE OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

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FLUCTUATIONS IN AGE COMPOSITION AND GROWTH RATE OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

Ross V. Bulkley
Fishery Research Biologist

RESEARCH REPORT 54

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ABSTRACT

Age composition, growth rate, and year-class strength of Yellowstone Lake cutthroat trout from collections made in 1948 and from 1950 to 1959 are analyzed to relate total catch to changes in age composition and growth rate. Catch increased from 200,015 cutthroats in 1950 to 393,467 in 1959. An increase in growth rate of fish fully recruited to the fishery and a decrease in percentages of fish belonging to age groups VI and VII are attributed to an increase in fishing pressure. Mean age of the catch varied with year-class strength, but was essentially unaffected by changes in size of the catch. Mean length of the catch has remained high, suggesting that production is more efficient now than in past years. Maximum equilibrium yield may be near.

If the catch continues to increase at the present rate, it may become excessive within the next few years.

FLUCTUATIONS IN AGE COMPOSITION AND GROWTH RATE OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

By Ross V. Bulkley, *Fishery Research Biologist*

Rocky Mountain Sport Fishery Investigations
Logan, Utah.

The population of cutthroat trout, *Salmo clarki lewisi*, in Yellowstone Lake has been recognized for many years as one of the last remaining large populations of pure cutthroat trout.[1] Most other lake populations of cutthroat have been contaminated by repeated introduction of rainbow trout or other subspecies of cutthroat. Several studies have been conducted in an effort to protect this important recreational resource from overexploitation and depletion. After World War II the National Park Service recognized the need for further research to determine the effect of the expected increase in fishing pressure on the lake. At its request, the U.S. Fish and Wildlife Service commenced a new study of the fishery in 1950.

Cope (1958) summarized studies made before 1958 at Yellowstone Lake. Little information on the age composition and growth rates of the population, a prerequisite to management, was presented in these studies owing to difficulty in aging cutthroat trout. Brown and Bailey (1952) found that the age group I cutthroat from Yellowstone Lake can be divided into three groups: (1) those that are completely scaled before their first winter of life and show annuli on all scales; (2) those that pass the first winter partially scaled and have some scales with annuli and some without; (3) those that have no scales during their first winter and thus lack the initial annulus on all scales. Growth studies by Madsen [2] and by Willoughby (1951) did not consider this complication, and many of their fish were older than the assigned age.

Laakso and Cope (1956) continued the study of scale and annulus formation and established criteria for distinguishing cutthroat scales which do not exhibit the initial annulus.

This paper summarizes data collected in 1948 and from 1950 to 1959 on the cutthroat trout of Yellowstone Lake and covers (1) growth rates, (2) age composition of fish in the spawning runs and catch, and (3) fluctuations in growth rate and age composition in relation to size of the catch.

I am indebted to Drs. Ralph Hile, Oliver B. Cope, and Norman G. Benson of the U.S. Fish and Wildlife Service and Dr. Kenneth D. Carlander of Iowa State University for their suggestions on analysis of the data. Much of the scale reading was done by Dr. Martin Laakso, formerly an employee of the U.S. Fish and Wildlife Service.

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[1] Other fish in Yellowstone Lake are longnose sucker *Catostomus catostomus* (Forster); smallfin redside shiner, *Richardsonius balteatus hydrophlox* (Cope); lake chub, *Hybopsis plumbea* (Agassiz); longnose dace, *Rhinichthys cataractae* (Valenciennes).

[2] Age and growth of the cutthroat trout (*Salmo lewisi*) in Yellowstone Lake, Wyoming, by M. J. Madsen. Unpublished report, U.S. Fish and Wildlife Service, 1940. 12 pp.

SCALE COLLECTION AND READING

Scale collections from fish in five spawning runs of Yellowstone Lake and in two segments of the fishery were studied for estimates of age composition and growth rates. Scale samples from spawning runs were obtained from fish captured in traps at Arnica, Pelican, Clear, Chipmunk, and Grouse Creeks (fig. 1). Scale samples from the fishermen's catches were collected from Fishing Bridge and West Thumb docks. Collections from the fishery were made periodically throughout the summer. Samples of the catch in the West Thumb area were obtained from rowboat and guideboat fishermen who used the West Thumb dock. Fish landed at this dock were caught either in West Thumb or in the west portion of Yellowstone Lake down to and occasionally including the South Arm. The catch from the Fishing Bridge area was sampled by obtaining data from fish landed at Fishing Bridge dock. Anglers operating from this dock fished both the upper 300 yards of the Yellowstone River and the lake proper. Guideboats which operated from Fishing Bridge until 1953 occasionally landed fish from the south arms of the lake as well as from less remote areas.

Scales from approximately 300 fish per year were selected from each sample for reading. Before 1959 each 5-millimeter length group was represented in the subsample according to the number of fish in

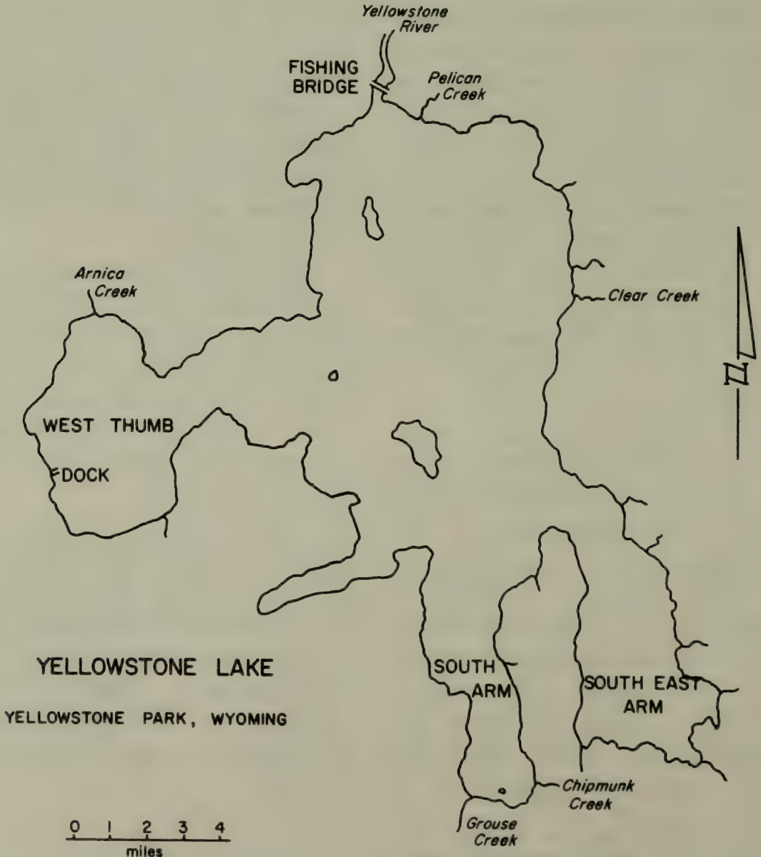

FIGURE 1.—Location of scale-collection stations at Yellowstone Lake.

that length group. Age composition of the subsample was thus representative of the total sample. In 1959 five fish were selected from each length group regardless of the number of fish in the group. Age composition of the total sample was then determined by expanding age composition of the five fish from each length group to include all fish in that group. The 1959 method provided essentially the same degree of accuracy in age and growth estimates with a smaller number of scale readings. In both methods, determination of age composition was based on many more fish than the number actually aged; some samples contained several thousand fish. Scale samples were also taken from small trout collected by seines, gill nets, and downstream traps to obtain data for determining the body-scale relation.

Scales were collected from the region between the dorsal fin and the lateral line. After cleaning, the scales were mounted in glycerin-gelatin or sodium silicate for viewing. Examination of the scales was made with a standard scale-viewing microprojector at a magnification of 115 diameters. All scales were read twice, and periodic checks by other scale readers were used to reduce error. Fish were aged according to criteria established by Laakso and Cope (1956) for the identification of scales which lack the first annulus. By close adherence to these criteria, errors in identifying retarded scales were kept to a minimum. Some subjectivity was necessary in scale interpretation because all gradations of the criteria were found. Approximately 3 percent of the fish in each sample were discarded because their scales were of questionable age. The discarded fish were from all size groups, so estimates of age distribution were not affected.

The terms "normal" scales and "retarded" scales are extended in this paper to include fish having those types of scales. A fish which formed an annulus during its first year is termed a normal fish, while a fish which did not form an annulus until its second year is referred to as a retarded fish (Laakso, 1955). Normal fish are the large yearlings and retarded fish are the small yearlings of a year class.

Back calculations of length were determined from mean scale measurements of each age group with the aid of a nomograph constructed from a plotted body-scale relation (appendix A).

GROWTH CALCULATIONS

Calculated lengths

Length calculations of Yellowstone Lake cutthroat were complicated by Lee's phenomenon (appendix B) and the presence of retarded scales in the samples. Lee's phenomenon is the decrease in calculated lengths as the age of the fish whose scale is used increases—the older the fish, the lower the calculated lengths. A correction was necessary to reduce the effect of this phenomenon (appendix C). Comparisons of growth rates among areas and among years were essentially unaffected by Lee's phenomenon, and no corrections were made for these comparisons. Varying percentages of retarded scales in the samples made detection of slight changes in growth rate difficult. The problem was most pronounced in comparing growth increments among areas because percentages of retarded scales in samples from each section of the lake differed. Comparisons among years were most accurate from samples collected in a single area. Retarded scales ceased to be a problem in growth comparisons by the end of the fifth year of life, as growth compensation is completed at that age (appendix D). Since growth rates of males and females were not significantly different, sexes were combined for all comparisons.

Differences in growth among areas

Cope (1953) demonstrated distinct differences in mean size of fish from various areas of Yellowstone Lake (table 1). Fish from Chipmunk and Grouse Creek spawing runs were consistently larger than fish from the other runs examined. He suggested that the size difference was due to a difference in growth rate or in age composition. Mean increments of length of fish from different areas of the lake were compared to determine whether rate of growth actually varied in different segments of the population (table 2). Increments from the combined collections of 1953 to 1955 were used in the comparison. Fish from Chipmunk and Grouse Creeks appear to grow slightly faster than fish from the other streams when total increments are compared, but this difference is due to different percentages of normal fish in the samples (appendix C). Analysis of variance in growth rate revealed that the differences in growth rate among areas were negligible. An F value of 0.375 was not significant at the 0.05-percent confidence level. Fish of age groups IV to VI grew at about the same rate in all areas during the period, even though discrete spawning groups in the lake have been recognized (Cope, 1957b).

Difference in growth among years

Comparisons of growth rates in different calendar years were made to determine whether a trend could be detected. Collections from 1948 to 1959 at West Thumb and Fishing Bridge docks were used in the comparisons. These two segments of the fishery sampled fish from many of the major spawning runs in the lake. Increments of length added from 1943 to 1958 are presented in tables 3 and 4.

Differences between calendar years were analyzed by the method described by Hile (1936) using percentage deviation from the mean increment of growth. A slight variation was made in Hile's method owing to the wide range in number of fish in different age groups. The number of fish in age groups II and VI was relatively small in all samples. A few fish in these age groups exerted as much influence on the yearly mean as several hundred fish in age groups IV and V. In order to include age groups II and VI in the calculations, the mean percentage deviation for a calendar year was obtained by weighting each percentage deviation by number of fish in the age group it represented. A more accurate estimate of relative growth was obtained in this way.

TABLE 1.—*Mean total lengths, at capture, of cutthroat trout from Yellowstone Lake spawning runs and fishery, 1949–59*

[In millimeters; data before 1952 from Cope (1953)]

Year	Lengths in spawing runs					Lengths in fishery	
	Arnica	Pelican	Clear	Chipmunk	Grouse	Fishing Bridge	West Thumb
1949	----------	[1] 344.7	----------	[1] 375.3	----------	----------	----------
1950	367.6	364.3	----------	378.6	----------	358.9	338.2
1951	362.8	363.1	----------	385.7	378.9	349.6	337.8
1952	353.5	355.9	----------	[1] 373.2	374.7	338.1	323.6
1953	352.9	358.3	368.6	396.9	376.6	350.8	331.8
1954	346.5	364.5	372.9	388.3	388.0	339.4	332.0
1955	355.4	362.2	366.0	391.1	392.9	[1] 347.2	[1] 339.0
1956	354.2	363.7	----------	----------	----------	352.3	344.0
1957	369.7	378.8	385.6	390.5	----------	353.2	353.3
1958	389.5	362.7	390.8	392.1	386.4	[1] 357.6	[1] 358.0
1959	368.7	379.9	391.3	387.2	382.0	355.5	353.2

[1] Biased estimate.

TABLE 2.—*Mean length increments of cutthroat from five spawning streams of Yellowstone Lake, combined collections of 1953 to 1955*

[In millimeters; number of fish in parentheses]

Age group and year of life	Mean increments of length by stream				
	Arnica	Pelican	Clear	Chip-munk	Grouse
Age group VI:					
Year 6	52	49	51	52	46
Year 5	91	87	134	82	76
Year 4	79	90	40	85	90
Year 3	108	100	86	94	91
Year 2	34	43	59	58	61
Year 1	44	44	45	43	46
	(13)	(33)	(21)	(90)	(22)
Age group V:					
Year 5	79	83	75	77	66
Year 4	79	84	80	86	96
Year 3	104	96	80	91	85
Year 2	40	50	75	71	83
Year 1	44	43	44	45	48
	(243)	(315)	(140)	(236)	(135)
Age group IV:					
Year 4	94	89	101	109	118
Year 3	101	106	80	74	87
Year 2	67	60	99	107	90
Year 1	45	44	45	46	48
	(386)	(574)	(301)	(321)	(178)

TABLE 3.—*Annual increments of growth in length of fish landed at West Thumb dock, 1948–59*

[In millimeters. Each diagonal row gives the growth history of an age group, belonging to the year class indicated by the year of first-year growth and captured in the calendar year following the one for which the last increment is given. Sample size of each age group in parentheses immediately below the first-year increment]

Age group and year of life	Growth increment in calendar year—															
	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958
Age group VI:																
Year 6	------	------	------	------	------	------	51	55	52	44	50	62	------	52	55	------
Year 5	------	------	------	------	------	98	95	88	95	90	78	------	70	102	------	------
Year 4	------	------	------	------	62	72	75	70	70	61	------	97	55	------	------	------
Year 3	------	------	------	88	84	115	79	95	84	------	98	88	------	------	------	------
Year 2	------	------	37	26	26	45	22	36	------	47	56	------	------	------	------	------
Year 1	------	43	43	47	44	43	43	------	43	44	------	------	------	------	------	------
	------	(17)	(8)	(18)	(17)	(9)	(9)	------	(6)	(4)	------	------	------	------	------	------
Age group V:																
Year 5	------	------	------	------	85	------	70	76	76	80	85	81	72	85	83	82
Year 4	------	------	------	70	------	87	82	75	72	73	76	82	84	90	98	------
Year 3	------	------	102	------	98	91	86	90	103	94	90	96	99	114	------	------
Year 2	------	51	------	48	49	51	45	27	45	60	47	52	53	------	------	------
Year 1	42	------	51	44	44	43	43	43	46	43	44	45	------	------	------	------
	(10)	------	(129)	(78)	(111)	(70)	(88)	(70)	(65)	(105)	(85)	(16)	------	------	------	------
Age group IV:																
Year 4	------	------	------	------	85	------	87	84	82	90	88	96	89	91	104	106
Year 3	------	------	------	113	------	99	98	96	103	101	95	82	99	104	81	------
Year 2	------	------	56	------	61	63	65	66	59	63	85	75	65	89	------	------
Year 1	------	43	------	46	45	47	46	45	44	49	43	44	44	------	------	------
	------	(40)	------	(105)	(149)	(189)	(155)	(200)	(145)	(135)	(137)	(120)	(56)	------	------	------
Age group III:																
Year 3	------	------	------	------	------	------	86	101	104	82	93	93	97	87	85	85
Year 2	------	------	------	------	------	89	88	97	97	83	90	108	97	104	102	------
Year 1	------	------	------	------	45	49	51	49	48	46	53	44	44	46	------	------
	------	------	------	------	(145)	(124)	(166)	(107)	(130)	(118)	(89)	(39)	(23)	(28)	------	------
Age group II:																
Year 2	------	------	------	------	------	------	122	------	113	127	128	107	------	------	------	140
Year 1	------	------	------	------	------	50	------	50	56	50	49	------	------	------	48	------
	------	------	------	------	------	(27)	------	(14)	(7)	(28)	(25)	------	------	------	(4)	------

TABLE 4.—*Annual increments of growth in length of fish landed at Fishing Bridge dock, 1948–59*

[In millimeters. Each diagonal row gives the growth history of an age group, belonging to the year class indicated by the year of first-year growth and captured in the calendar year following the one for which the last increment is given. Sample size of each age group in parentheses immediately below the first-year increment]

Age group and year of life	Growth increment in calendar year—															
	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958
Age group VI:																
Year 6	------	------	------	------	------	------	54	52	57	44	------	52	------	65	46	------
Year 5	------	------	------	------	------	82	79	80	88	------	86	------	97	93	------	------
Year 4	------	------	------	------	65	75	68	77	------	73	------	92	77	------	------	------
Year 3	------	------	------	94	84	83	96	------	93	------	112	93	------	------	------	------
Year 2	------	------	44	36	48	54	------	38	------	22	45	------	------	------	------	------
Year 1	------	44	43	43	43	------	44	------	43	44	------	------	------	------	------	------
	------	(29)	(48)	(16)	(22)	------	(26)	------	(7)	(12)	------	------	------	------	------	------
Age group V:																
Year 5	------	------	------	------	93	------	74	82	75	80	75	79	77	76	68	81
Year 4	------	------	------	45	------	84	68	78	74	93	77	78	84	99	104	------
Year 3	------	------	78	------	92	102	94	95	80	94	91	99	92	106	------	------
Year 2	------	63	------	54	37	40	50	56	57	58	50	55	51	------	------	------
Year 1	44	------	46	43	45	44	44	45	44	43	44	46	------	------	------	------
	(4)	------	(108)	(129)	(110)	(126)	(90)	(123)	(80)	(110)	(113)	(20)	------	------	------	------
Age group IV:																
Year 4	------	------	------	------	73	------	69	80	88	69	85	86	87	94	96	82
Year 3	------	------	------	83	------	87	97	92	112	101	109	87	99	96	100	------
Year 2	------	------	80	------	60	47	72	76	60	57	83	76	74	54	------	------
Year 1	------	45	------	45	43	45	45	45	46	44	44	45	44	------	------	------
	------	(33)	------	(110)	(121)	(184)	(110)	(69)	(80)	(103)	(191)	(136)	(39)	------	------	------
Age group III:																
Year 3	------	------	------	------	120	------	91	120	81	96	89	95	86	94	86	98
Year 2	------	------	------	67	------	86	48	96	108	99	91	117	87	90	81	------
Year 1	------	------	50	------	47	44	47	44	44	44	49	44	44	44	------	------
	------	------	(11)	------	(38)	(48)	(145)	(76)	(43)	(60)	(44)	(44)	(29)	(24)	------	------
Age group II:																
Year 2	------	------	------	------	------	------	88	------	101	80	159	150	104	140	------	113
Year 1	------	------	------	------	------	46	------	56	47	49	49	50	47	------	52	------
	------	------	------	------	------	(3)	------	(14)	(21)	(17)	(9)	(2)	(5)	------	(15)	------

Percentage deviations were calculated for the period 1946 to 1958 (table 5). Although the data included samples from 1943 to 1958, the extreme years were represented by only a few fish. The data for 1957 and 1958 were included because of their importance, but the actual percentage values for these two years are not as accurate as those for the earlier years. Data from 1943 to 1945 were not included.

Calendar years 1947 through 1953 appear to be the poorest growth years, and 1954 and 1956 to 1958 the best growth years, for fish in the West Thumb area. Samples from Fishing Bridge also indicate that the growth rate before 1950 was slower than in later years. Fishing Bridge showed rapid growth from 1953 to 1957. Samples from both areas agreed that years before 1950 and 1952 were poor growth years and that 1954, 1956, 1957, and 1958 were good growth years. The cyclic nature of the fluctuations and the trend toward a more rapid growth rate are notable. Analysis of growth rate by age group revealed that this increased growth rate occurred among fish of age groups IV, V, and VI only. Rate of growth of age groups II and III fish fluctuated independently of the older age groups, and no upward trend was obvious (fig. 2). Growth of younger fish is apparently controlled by other factors than those influencing fish 4 years of age and older.

Growth comparisons among year classes also suggest that the growth rate increased after 1953 (table 6). Collections of Arnica Creek spawners from 1951 to 1959 were analyzed for variations in growth rate among year classes. Year class 1949 was the slowest growing of all year classes produced from 1945 to 1956. Year classes 1945, 1946, 1947, 1950, 1952, 1953, and 1954 showed only minor deviations in mean growth rate for the period. Year classes 1955 and 1956 showed a decided increase in growth rate although those year classes were based on a single age group.

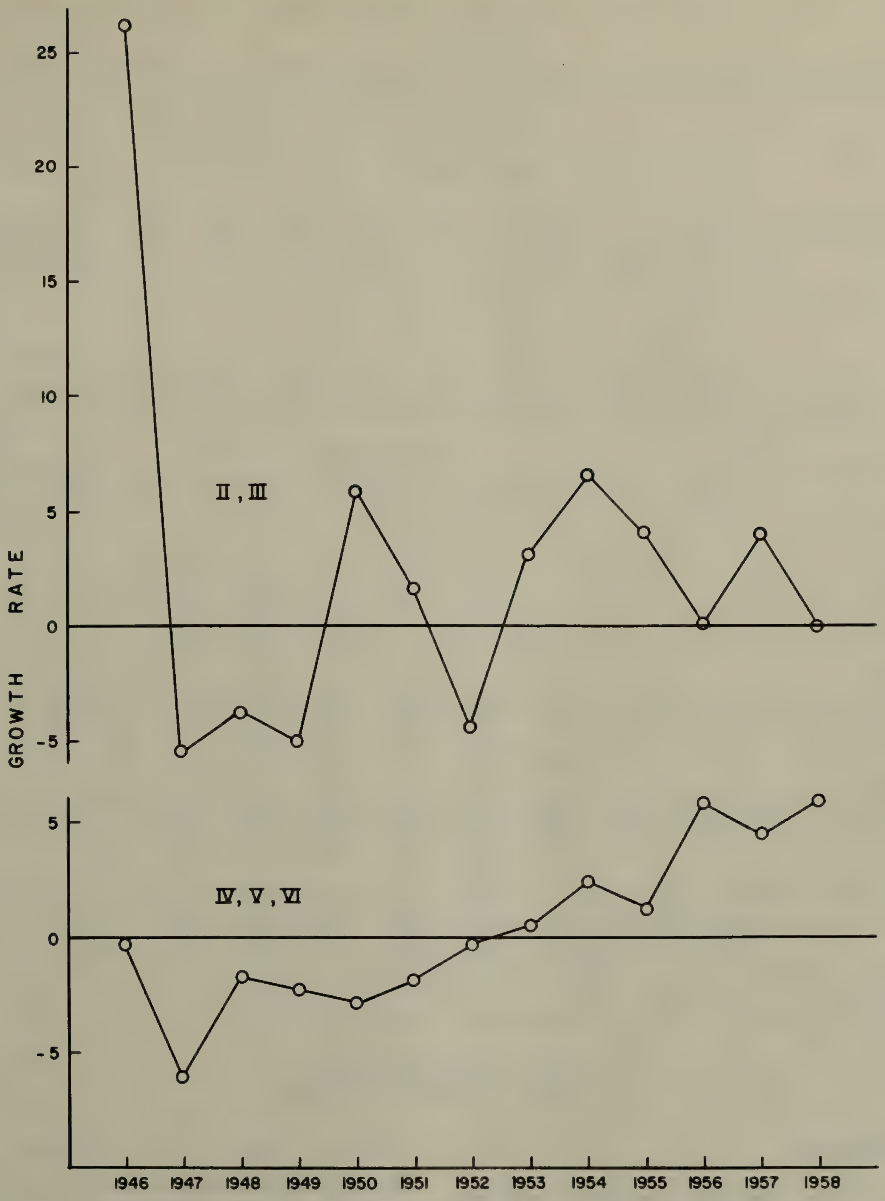

Figure 2.—Percentage deviation in growth rate by age group of fish from combined West Thumb and Fishing Bridge collections.

TABLE 5.—*Mean percentage deviation of growth in length of cutthroat from Fishing Bridge and West Thumb fisheries, 1946–58*

[Collections from 1948 and 1950 to 1959. Each percentage deviation is weighted according to sample size given in tables 3 and 4 to obtain mean for the calendar year for each area. Mean is the unweighted mean of West Thumb and Fishing Bridge percentages]

Area	Mean percentage deviation from average growth in calendar year—												
	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958
Fishing Bridge dock	−0.93	−8.06	−5.25	−1.59	2.55	0.41	−0.97	2.76	4.68	3.73	3.10	2.33	1.72
West Thumb dock	1.30	−3.07	0.97	−3.31	−3.60	−1.53	−1.66	−0.71	1.37	−0.55	6.73	4.14	6.92
Mean	0.12	−6.37	−2.78	−2.50	−1.04	−0.61	−1.32	1.25	2.63	1.39	4.80	3.21	4.08

TABLE 6.—*Growth comparison among year classes of Arnica Creek spawners, 1945–56*

[In millimeters. Number of fish in parentheses. Mean percentage deviation is the unweighted mean for the year class]

Age group and year of life	Increments of growth by year class of—											
	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
Age group VI:												
Year 6	49	44	--------	58	--------	--------	40	53	--------	--------	--------	--------
Year 5	86	98	--------	83	--------	--------	95	83	--------	--------	--------	--------
Year 4	87	66	--------	80	--------	--------	93	64	--------	--------	--------	--------
Year 3	103	91	--------	101	--------	--------	92	100	--------	--------	--------	--------
Year 2	47	77	--------	--------	--------	--------	56	46	--------	--------	--------	--------
Year 1	43	43	--------	--------	--------	--------	44	49	--------	--------	--------	--------
	(31)	(5)	--------	(7)	--------	--------	(7)	(8)	--------	--------	--------	--------
Age group V:												
Year 5	--------	82	76	78	79	81	94	80	74	88	--------	--------
Year 4	--------	88	97	85	73	84	66	93	96	100	--------	--------
Year 3	--------	95	100	92	98	95	94	101	127	94	--------	--------
Year 2	--------	50	51	49	49	48	42	45	25	49	--------	--------
Year 1	--------	43	44	44	44	44	43	44	43	44	--------	--------
	--------	(96)	(100)	(60)	(94)	(89)	(52)	(78)	(53)	(35)	--------	--------
Age group IV:												
Year 4	--------	--------	89	94	93	87	96	89	100	95	110	--------
Year 3	--------	--------	104	101	103	107	101	91	99	92	89	--------
Year 2	--------	--------	70	71	65	64	69	75	77	85	83	--------
Year 1	--------	--------	43	43	44	45	46	47	45	46	45	--------
	--------	--------	(135)	(152)	(122)	(74)	(190)	(91)	(77)	(29)	(63)	--------
Age group III:												
Year 3	--------	--------	--------	114	95	94	84	109	99	71	--------	96
Year 2	--------	--------	--------	97	100	97	97	99	83	106	--------	116
Year 1	--------	--------	--------	45	45	49	47	49	47	45	--------	51
	--------	--------	--------	(34)	(51)	(23)	(23)	(26)	(16)	(10)	--------	(10)
Mean percentage deviation	−1.08	1.55	1.07	1.71	−2.22	−1.13	−2.07	−0.24	−1.59	1.28	5.00	8.47

AGE COMPOSITION

Sex differences

Several authors have reported an uneven sex ratio among adult cutthroat trout. Ball and Cope (1961) reported a preponderance of females in the spawning runs of Yellowstone Lake and quoted a male-to-female sex ratio from combined data of 1:1.47. The ratio was 1 male to 1.62 females in the fishery. They found no explanation for this uneven sex ratio, but concluded that it was not due to an uneven harvest of males.

Bjornn (1957) found a similar preponderance of females in the spawning runs and sport catch of Priest and Upper Priest Lakes. He stated that the males, which matured at a smaller size, may have passed through the weir of the traps and thus may not have been included in the count of spawners. He did not explain the unbalanced sex ratio in the catch.

Samples from the Arnica Creek spawning run and the West Thumb fishery were examined to determine the sex ratio in each age group (fig. 3). Females were

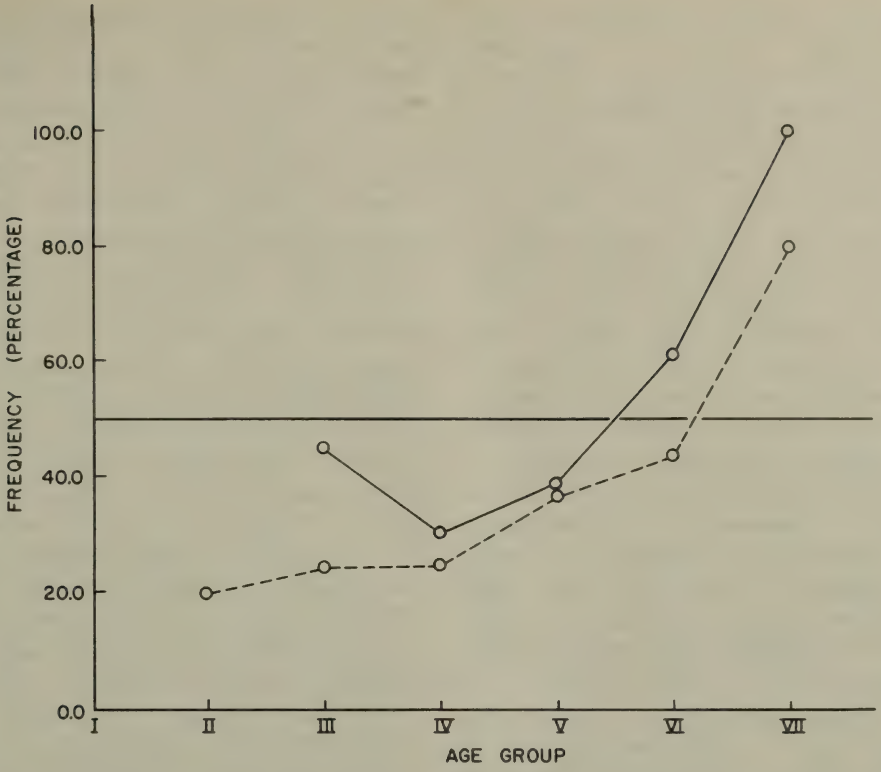

FIGURE 3.—Percentage of males in each age group of Arnica Creek spawners (solid line) collected from 1951 to 1957 and of West Thumb catch (broken line) collected from 1950 to 1956.

more abundant in the Arnica Creek spawning runs of 1951 to 1957 up to age V; at age VI and VII the males were predominant. In the West Thumb catch from 1950 to 1956, males were less abundant than females up to age VI, and more abundant at age VII. Comparison, by age group and sex, of 272 fish captured in gill nets from all areas of the lake during 1958 showed the following percentages of males in each age group: Age II, 35.5; age III, 37.9; age IV, 39. 5; age V, 51. 4; and age VI, 50.0 (2 fish). The sex ratio of the gill-net sample was not as unbalanced as that in the spawning runs and the catch, but it also indicated that there may be a differential mortality between sexes. Van Oosten (1929) found a similar situation for lake herring—females more numerous than males in age groups I and II, males more numerous in the older age groups. He suggested that one explanation could be that females mature sooner than males and hence are more abundant in the younger age groups as spawners. Difference in age at maturity of cutthroat should not affect the sex ratios obtained from gill-net samples or from the fishery samples, as males and females in each age group were the same size. The reason for the uneven sex ratio is not known, but the condition appears to be characteristic of several cutthroat populations.

Composition of spawning runs

Fish in Yellowstone Lake spawning runs vary in age from 3 to 7 years. Only the fastest-growing fish become sexually mature at age III, and data presented later suggest that recruitment to the runs is not complete until age V. From 77 to 98 percent of the spawning runs examined from 1951 to 1959 were composed of fish of age groups IV and V, with age group IV usually most abundant. Age group VII fish were rare in all samples.

Considerable differences in age composition were noticeable in spawning runs throughout the lake. Cope (1953) commented on the greater mean length of spawners from Chipmunk and Grouse Creeks as compared with those in the northern streams. The present study determined that some of the difference in mean length was due to a larger proportion of normal fish in the older age groups, but a difference in age composition, as suggested, was the main source of the variation. Examination of the collections from 1953 to 1955 revealed that Grouse and Chipmunk Creeks had the oldest populations of spawners (fig. 4). Little difference was apparent in age composition of spawners from Arnica, Pelican, and Clear Creeks. Difference in the age composition can be more readily seen by comparing the percentage of old fish in the samples for the 3-year period. Percentages of the samples composed of fish belonging to age group V and older for the five streams were as follows: Arnica, 35.9; Pelican, 36.7; Clear, 34.5; Grouse, 46.1; and Chipmunk, 50.9. Both the mean total lengths and the mean age of Chipmunk Creek spawners were higher than for spawners from other streams.

Dominant year classes were difficult to detect from the age composition of the spawning runs, owing to the narrow range in age of spawners. Hence, any year class that contributed strongly in two or more years was considered a strong year class. The 1949 year class provided the majority of spawners for both 1953 and 1954 in Arnica, Pelican, and Grouse Creeks (table 7). In the Clear Creek samples the 1951 year class provided 75.9 percent of the 1955 spawning run in that stream, but no other year class appeared dominant. The 1947 year class of Chipmunk Creek provided above-average percentages of fish in the 1952, 1953, and 1954 runs.

Completeness of the data from Arnica Creek on age composition and size of

TABLE 7.—*Percentage of fish in different age groups in five spawning runs of Yellowstone Lake, 1951–59*

Area and year	Number of fish in run	Number of fish aged	Percentage in age group—				
			III	IV	V	VI	VII
Pelican Creek:							
1953	12,418	156	8.6	58.3	29.6	2.3	1.2
1954	10,340	270	3.4	55.0	37.0	4.4	0.2
1955	12,400	563	6.5	58.2	32.4	2.5	0.4
1956 [1]	7,000	325	5.5	59.7	32.4	2.2	0.2
Clear Creek:							
1953	10,323	145	0.0	60.7	35.2	4.1	0.0
1954	3,161	192	0.0	58.3	33.3	7.8	0.6
1955	7,929	133	5.3	75.9	18.8	0.0	0.0
Chipmunk Creek:							
1952	9,989	94	0.0	56.4	39.4	4.2	0.0
1953	7,836	168	0.0	40.5	38.1	20.8	0.6
1954	5,371	227	0.4	40.5	39.2	15.9	4.0
1955	3,880	267	1.2	60.2	31.1	7.1	0.4
Grouse Creek:							
1952	6,949	212	0.0	55.2	43.4	1.4	0.0
1953	7,441	180	4.4	63.4	28.9	3.3	0.0
1954	3,756	71	0.0	24.4	59.4	13.5	2.7
1955	(2)	91	0.0	50.6	42.8	6.6	0.0
Arnica Creek:							
1951	4,755	298	11.4	45.3	32.2	10.4	0.7
1952	3,522	308	16.6	49.3	32.5	1.6	0.0
1953	4,524	207	11.1	58.9	29.0	1.0	0.0
1954	6,200	197	11.5	37.5	47.5	3.5	0.0
1955	4,700	309	8.4	61.5	28.8	1.3	0.0
1956	3,912	163	9.8	55.8	31.9	2.5	0.0
1957	3,812	339	2.9	41.0	53.7	2.4	0.0
1958	4,041	91	1.9	30.7	59.9	6.5	1.0
1959	1,755	110	4.3	62.6	32.2	0.9	0.0

[1] Poor sample, owing to high water.
[2] Count not made.

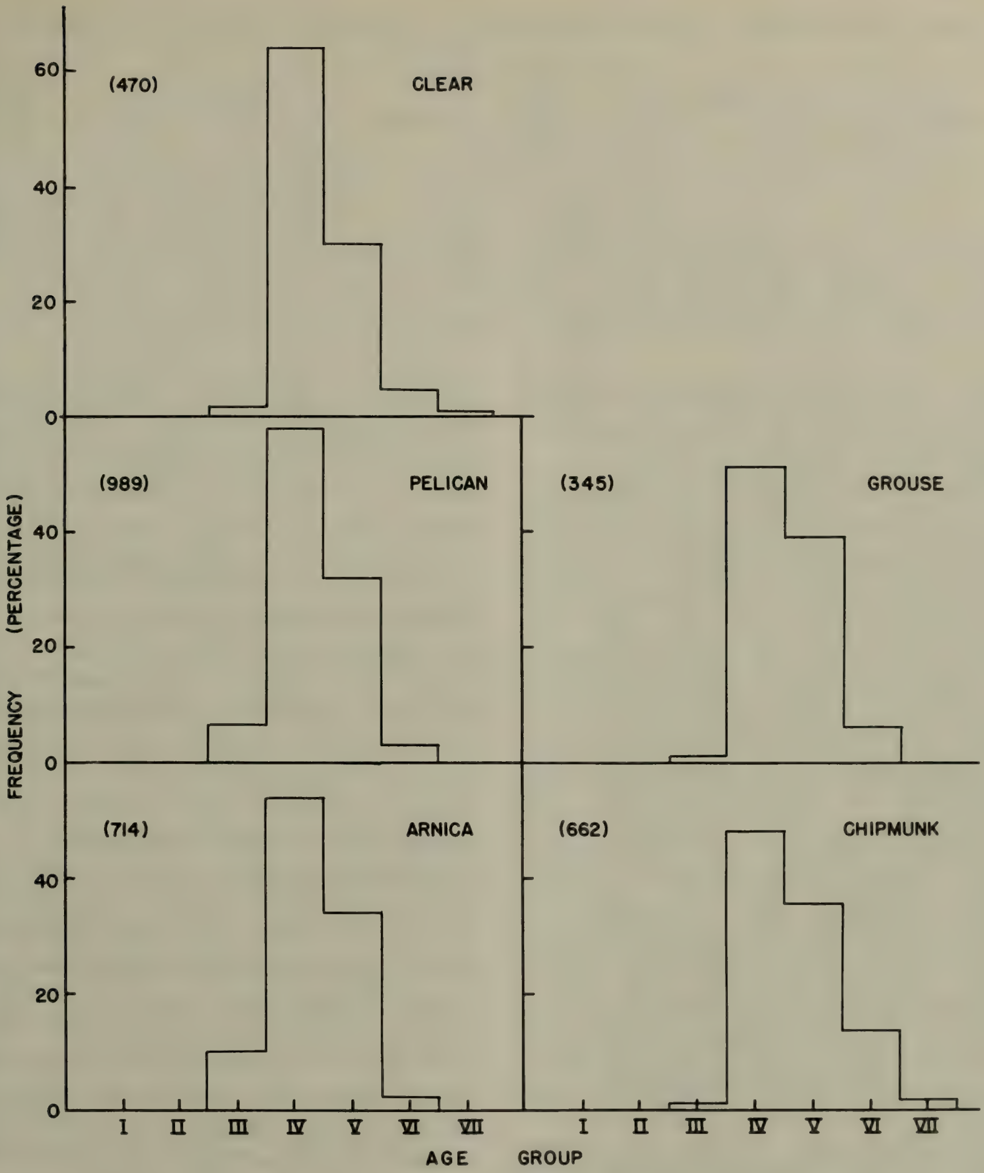

FIGURE 4.—Age composition of five Yellowstone Lake spawning runs from combined collections of 1953 to 1955. Number of fish in parentheses.

spawning runs provided a more accurate method than age composition alone for determining year-class strength for that population (table 8). Annual counts of the spawning runs from 1951 to 1959 in Arnica Creek were combined with age composition to determine relative contribution of each year class. Horizontal summation of fish numbers in the table provides an accurate estimate of year-class strength, even though the number of repeat spawners is unknown and some fish were undoubtedly counted several times. By this method the 1949 year class was the largest during the period; it contributed 6,256 spawners to the Arnica

TABLE 8.—*Contribution of different year classes to Arnica Creek spawning runs, 1944–56*

[Data from 1951 to 1959 collections]

Year class	Number of fish at age—					Total contribution
	III	IV	V	VI	VII	
1944	------	--------	--------	------	33	--------
1945	------	--------	--------	495	------	--------
1946	------	--------	1,531	56	------	--------
1947	------	2,154	1,145	45	------	--------
1948	542	1,736	1,312	217	------	3,807
1949	585	2,665	2,945	61	------	6,256
1950	502	2,325	1,354	98	------	4,279
1951	713	2,890	1,248	91	40	4,982
1952	395	2,183	2,047	263	------	4,888
1953	383	1,563	2,420	16	------	4,382
1954	111	1,241	565	------	------	--------
1955	77	1,099	--------	------	------	--------
1956	75	--------	--------	------	------	--------

Creek runs. Year classes 1949, 1951, and 1952 were strong in Arnica Creek; year classes 1948 and 1954 were weak.

Further comment should be made on the age composition of the spawning runs. Ball and Cope (1961) reported the results of tagging studies conducted at Yellowstone Lake from 1949 to 1953. They concluded that an average of only 1.22 percent of the spawning runs was composed of fish that had spawned before, and that the figure was always less than 10 percent. Cutthroat were reported to mature at age III or IV. In this paper the relatively large percentage of fish older than age IV in the spawning runs appears to disagree with the tagging data. For example, in Arnica Creek spawning runs from 1951 to 1959, the mean percentage of age V fish in the samples was 38.4 percent. An average of 3.6 percent was age group VI fish, which by itself is three times as large as the reported average percent of repeat spawners of all ages.

Two possible explanations for the apparent discrepancy are suggested. Most cutthroat trout mature at age IV in Yellowstone Lake, but some may not mature until age V. Delayed maturing of cutthroat has been reported from other areas. Bjornn (1957) found that the majority of cutthroat in Priest and Upper Priest Lakes, Idaho, reach maturity at age V; some were age VI before they spawned. He indicated that fish which matured at these late ages spent several years of slow growth as fingerlings in the spawning streams before descending to the lake. Few fingerlings spend more than 1 year in Yellowstone spawning streams before descending to the lake, so one would not expect many fish to need 5 years of growth to reach maturity. Another possibility is that tagging induced a differential mortality and made the percentage of repeat spawners appear to be less than normal. Ball and Cope (1961) found that tagging of spawners caused from 4 to 12 percent additional mortality while the fish were in the spawning streams. They were unable to determine whether this differential mortality continued after the spawning period.

Examination of the scales of spawning fish for indications of previous spawning was the only method available for determining possible reasons for the disagreement. Cutthroat spawning is usually associated with drastic scale resorption. After scale growth is resumed, the scars can often be separated from annuli. Scales of 58 fish of age group V from the 1957 Arnica Creek spawning run were examined for spawning checks. From the presence or absence of spawning marks it was apparent that 25 of the 58 fish or 43 percent, had spawned before; 4 fish were alternate-year spawners, 20 were consecutive-year spawners, and 1 appeared to be on its third spawning run. Forty-three percent of age V represented 12.4 percent of the spawning run. Six fish were placed in an "undecided" category, as the scale reader was not certain whether previous spawning had occurred. Of 27 fish which were classified as initial spawners, 3 had clipped fins indicating that they had spawned before. Hence, some fish obviously spawn without excessive scale deterioration, and the above method for determining repeat spawners is not completely reliable. There was sufficient confidence in the method, however, to conclude that some cutthroat in Yellowstone Lake spawn for the first time at age V, and that the percentage of repeat spawners in the runs is much higher than the tagging data suggest. Age group V in the spawning runs probably includes three classes of fish: consecutive spawners, alternate spawners, and a few initial spawners.

Composition of the catch

The catch in Yellowstone Lake from 1950 to 1959 was composed mostly of age groups IV and V (fig. 5). These were the same age groups which were predominant in the spawning runs. During this period, from 27 to 68 percent of the catch landed in different years at West Thumb and Fishing Bridge docks were age IV fish; from 10 to 41 percent belonged to age group V. Age IV was the most abundant age group in 1950, 1952, 1957, 1958, and 1959 at Fishing Bridge, and in all years of the period except 1950 at West Thumb. The 1959 collection had a higher percentage of age IV fish than any previous collection from the catch. Age V was proportionally larger in 1951, 1953, 1954, and 1955 at Fishing Bridge and in 1950 at West Thumb. Mean age of the West Thumb catch was considerably lower than mean age of the Fishing Bridge catch; the reason for this difference will be discussed later. Age VI fish were uncommon in the catch, especially in later years, and age VII fish were rare. Since 1955, no age group VII fish have been collected from the fishery. No age group VI fish were sampled during 1959 in the Fishing Bridge area, and only 0.1 percent (two fish) of the West Thumb sample were age VI. The small percentages of age II fish in the catch indicate that only the larger fish are recruited at that early age. Age group III contributed significant numbers of fish to the catch in all years, especially in the West Thumb area.

Year-class strengths were determined from the catch by two methods. Percentage age composition of samples was first compared for suggestion of year class strength. Any sharp deviation from average percentage contributions in a given age group suggests a relatively strong or weak year class. Hile (1954) discussed possible errors in using this method, but concluded that it probably provides the best evidence of relative year class strength. At Fishing Bridge the 1949 year class contributed heavily to the catch at age III, comprising 30.9 percent of the 1952 collection (table 9). The 1952 year class contributed heavily to the catch at age II (1954) and age IV (1956), and slightly more than average at age III (1955). Year class 1953 was abundant also in the 1957 and 1958 collections from Fishing Bridge. At West Thumb the 1947 year class contributed significantly to the catch at age III and VI; the 1952 and 1953 year classes contributed strongly at all ages. The 1955 year class comprised over half of the 1959 catch in both areas.

The completeness of the data collected in the 10-year period suggested that further analysis of year-class strength was merited. Adaptation by el Zarka (1959) of Hile's method of presenting relative growth rates was employed. This method is based on a series of comparisons of the relative contributions to the catch of two consecutive year classes. Data from table 9 will be used to illustrate the method. The 1946 year class at Fishing Bridge made up 37.9 percent of the 1950 collection at age IV, 36.3 percent of the 1951 collection at age V, and 3.4 percent of the

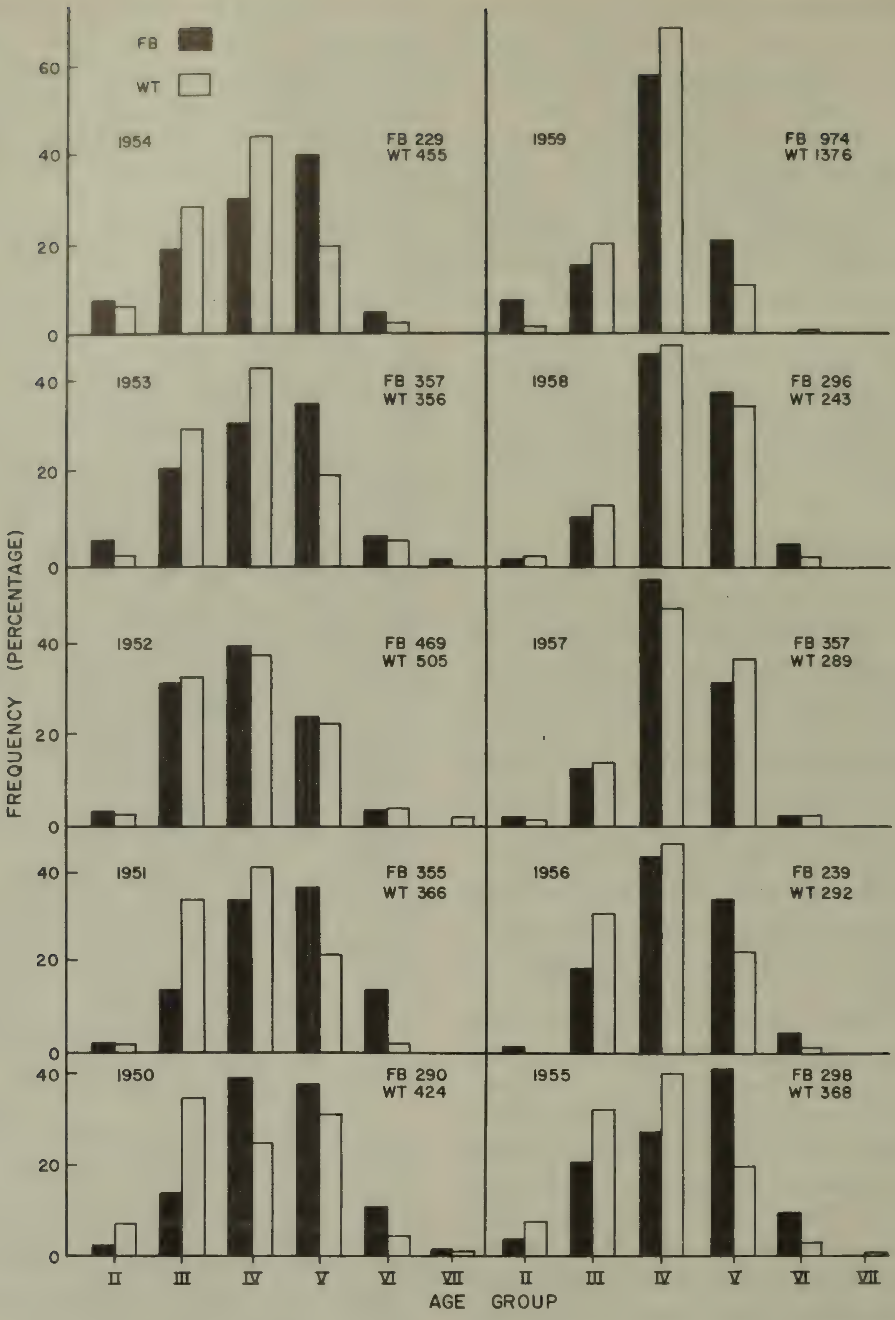

FIGURE 5.—Age composition of catch from Fishing Bridge and West Thumb areas. Collections from 1950 to 1959, with number of fish included.

TABLE 9.—*Age composition and year classes of Yellowstone Lake cutthroat, 1943–59*

[Data obtained from samples of the catch from 1950 to 1959]

Year of capture	Year class of—														
	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957
1950:															
Age group	VII	VI	V	IV	III	II	------	------	------	------	------	------	------	------	------
Percentage:															
Fishing Bridge (290 fish)	0.7	10.0	37.2	37.9	13.1	1.0	------	------	------	------	------	------	------	------	------
West Thumb (424 fish)	0.2	4.0	30.4	24.8	34.2	6.4	------	------	------	------	------	------	------	------	------
1951:															
Age group	------	VII	VI	V	IV	III	II	------	------	------	------	------	------	------	------
Percentage:															
Fishing Bridge (355 fish)	------	0.7	13.5	36.3	34.0	13.5	2.0	------	------	------	------	------	------	------	------
West Thumb (366 fish)	------	0.3	2.2	21.3	40.7	33.9	1.6	------	------	------	------	------	------	------	------
1952:															
Age group	------	------	VII	VI	V	IV	III	II	------	------	------	------	------	------	------
Percentage:															
Fishing Bridge (469 fish)	------	------	------	3.4	23.4	39.2	30.9	3.0	------	------	------	------	------	------	------
West Thumb (505 fish)	------	------	1.4	3.6	22.0	37.4	32.8	2.8	------	------	------	------	------	------	------
1953:															
Age group	------	------	------	VII	VI	V	IV	III	II	------	------	------	------	------	------
Percentage:															
Fishing Bridge (357 fish)	------	------	------	0.6	6.1	35.3	30.8	21.3	5.9	------	------	------	------	------	------
West Thumb (356 fish)	------	------	------	------	4.8	19.7	43.5	30.0	2.0	------	------	------	------	------	------
1954:															
Age group	------	------	------	------	------	VI	V	IV	III	II	------	------	------	------	------
Percentage:															
Fishing Bridge (229 fish)	------	------	------	------	------	4.4	39.3	30.1	18.8	7.4	------	------	------	------	------
West Thumb (455 fish)	------	------	------	------	------	2.0	19.3	44.0	28.5	6.2	------	------	------	------	------
1955:															
Age group	------	------	------	------	------	VII	VI	V	IV	III	II	------	------	------	------
Percentage:															
Fishing Bridge (298 fish)	------	------	------	------	------	------	8.7	41.3	26.9	20.1	3.0	------	------	------	------
West Thumb (368 fish)	------	------	------	------	------	0.3	2.5	19.0	39.4	32.1	6.8	------	------	------	------
1956:															
Age group	------	------	------	------	------	------	------	VI	V	IV	III	II	------	------	------
Percentage:															
Fishing Bridge (239 fish)	------	------	------	------	------	------	------	4.2	33.5	43.0	18.4	0.8	------	------	------
West Thumb (292 fish)	------	------	------	------	------	------	------	1.0	22.3	46.2	30.5	------	------	------	------
1957:															
Age group	------	------	------	------	------	------	------	------	VI	V	IV	III	II	------	------
Percentage:															
Fishing Bridge (357 fish)	------	------	------	------	------	------	------	------	2.0	30.8	53.5	12.3	1.4	------	------
West Thumb (289 fish)	------	------	------	------	------	------	------	------	2.1	36.3	47.4	13.5	0.7	------	------
1958:															
Age group	------	------	------	------	------	------	------	------	------	VI	V	IV	III	II	------
Percentage:															
Fishing Bridge (296 fish)	------	------	------	------	------	------	------	------	------	4.0	38.1	46.3	10.5	1.0	------
West Thumb (243 fish)	------	------	------	------	------	------	------	------	------	1.6	34.6	48.5	13.2	2.0	------
1959:															
Age group	------	------	------	------	------	------	------	------	------	------	VI	V	IV	III	II
Percentage:															
Fishing Bridge (974 fish)	------	------	------	------	------	------	------	------	------	------	------	17.4	54.4	20.0	8.2
West Thumb (1,376 fish)	------	------	------	------	------	------	------	------	------	------	0.1	10.4	68.0	20.2	1.3

1952 collection at age VI. Summation of the contributions at ages IV, V, and VI was 77.6 percent. Year class 1947 made the following contributions at the same ages: 34.0 percent of the 1951 collection at age IV, 23.4 percent of the 1952 collection at age V, and 6.1 percent of the 1953 collections at age VI. Total of these contributions was 63.5 percent. The percentage difference in strength of the two year classes was then determined in the following manner:

$$\frac{2\ (77.6-63.5)}{77.6+63.5}\times 100=-20.0.$$

Table 10 summarizes the comparisons and calculations which were made to rank all year classes. The percentage differences between years were first adjusted to give the relative position of each year class.

This adjustment was made by arbitrarily giving year classes 1943 at Fishing Bridge and 1944 at West Thumb a value of 0.0, and adding percentage differences of each succeeding comparison to it. This series of figures was then adjusted to a mean of 0.0 for all year classes covered to obtain the final rankings presented in the right-hand columns of table 10. The resulting relative year-class strength is shown graphically in figure 6.

Dominant year classes described by the above method were 1945, 1949, 1953, 1956, and 1957 for Fishing Bridge; weak year classes were produced in 1947, 1951, and 1954. Year classes 1944, 1947, 1952, 1953, 1956, and 1957 were strong in the West Thumb samples; year classes 1946 and 1954 were weak.

Changes in age composition of the catch as the summer progressed were noticeable and have been reported for the Fishing Bridge area in 1952 (Laakso and Cope, 1956). Composition of the combined collections from 1950 to 1956 from West Thumb and Fishing Bridge areas in the present paper agrees with the findings for 1952 (fig. 7). The number of fish of age groups II and III in the catch increased as the season progressed, owing to continual recruitment. The large percentage of age group V fish in the early catch at Fishing Bridge has been explained by the presence of large numbers of spawners, from both the lake and the river, which congregate near the lake outlet in the early part of the fishing season. As these spawners disperse later in the summer, the mean age of the catch drops. In the West Thumb area the percentage of age III fish was relatively high during the whole summer. The high catch of age III fish is explained by the fact that most of the early fishing pressure

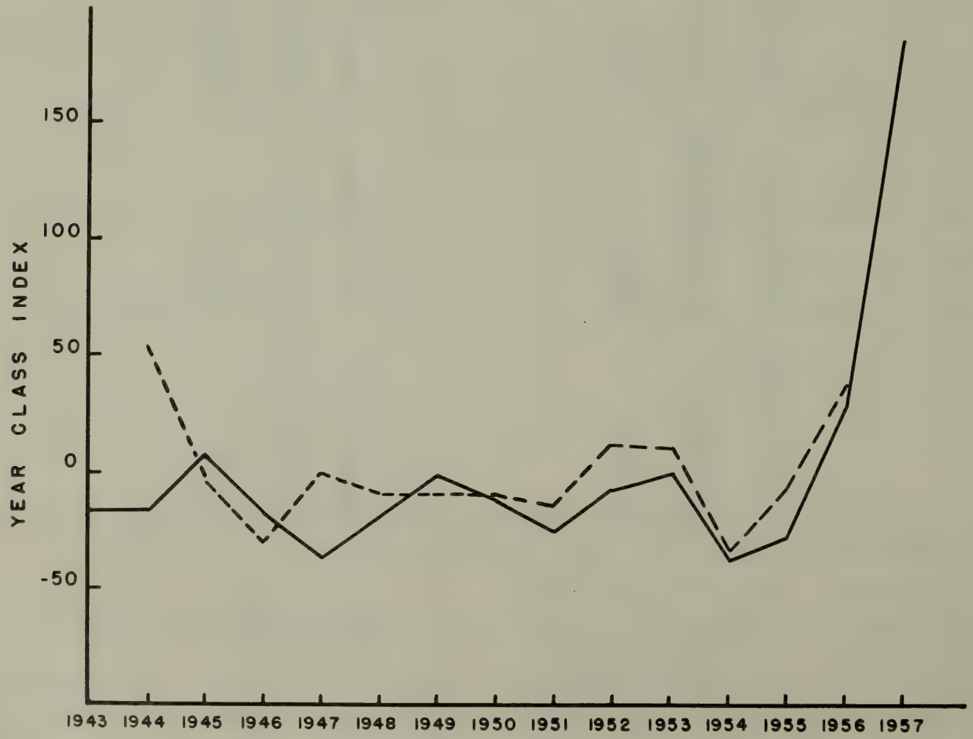

FIGURE 6.—Fluctuations in relative strength of year classes 1943 to 1957 of Yellowstone Lake cutthroat collected from Fishing Bridge dock (solid line) and West Thumb dock (broken line).

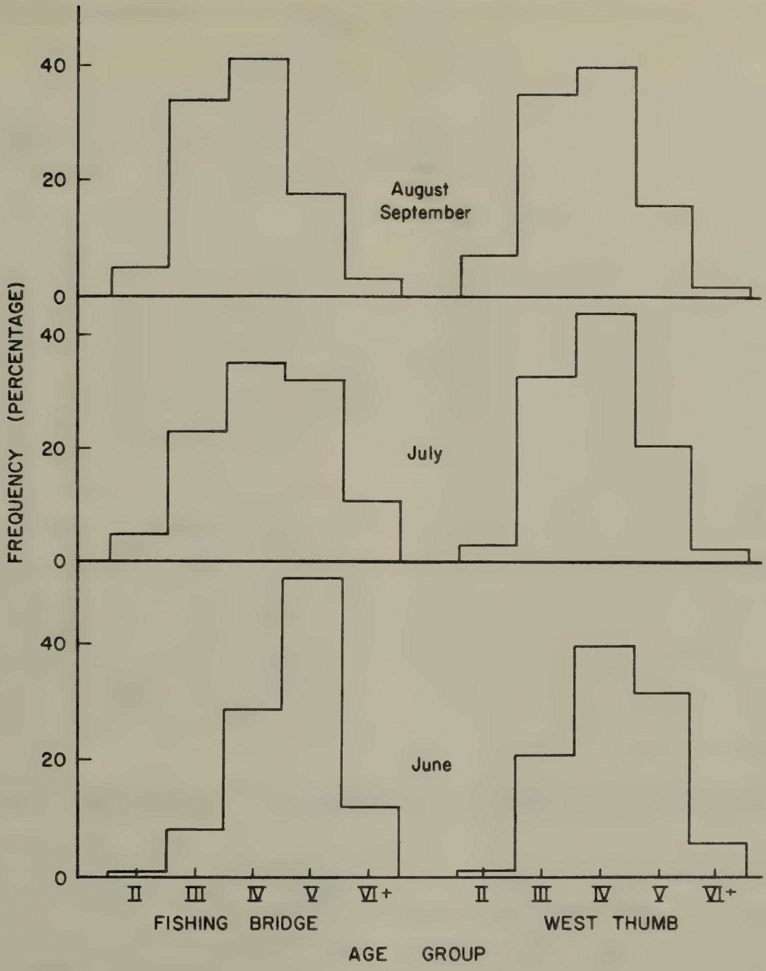

Figure 7.—Age composition of the catch from West Thumb and Fishing Bridge docks by month from June to September, collections of 1950 to 1956 combined.

in the West Thumb area is on nonspawners, so the younger fish which comprise most of this group are cropped heavily (Cope, 1957a). Later in the summer more age III fish are recruited into the fishery, and the age group continues to contribute to the creel. There are not as many older fish in the early West Thumb collections because that segment of the fishery is not concentrated over a big spawning population as is the Fishing Bridge fishery.

The great change in age composition as the fishing season progressed, particularly in the Fishing Bridge area, illustrated the importance of collecting samples throughout the summer for comparisons of age composition between years. A single collection for the year during June at Fishing Bridge dock would make the catch appear to consist almost entirely of large, mature fish. A sample taken in August would be very similar to the catch of much younger fish at West Thumb. Scale samples in this study were collected at regular intervals during the summer to avoid biased comparisons. One exception occurred in 1955 when scale collecting terminated on August 9 at Fishing Bridge dock.

TABLE 10.—*Data employed in determination of relative strength of year classes 1943 to 1957 of Yellowstone Lake cutthroat*

[See text for explanation of columns]

Year classes compared	Age groups included	Sum for first year	Sum for second year	Mean	Difference	Percentage difference between years	Relative strength of first year	Final rank of first year
Fishing Bridge dock:								
1943–1944	VII	0.7	0.7	0.7	0.0	0.0	0.0	−16.5
1944–1945	VI, VII	10.7	13.5	12.1	2.8	23.1	0.0	−16.5
1945–1946	V, VI	50.7	39.7	45.2	−11.0	−24.3	23.1	6.6
1946–1947	IV, V, VI	77.6	63.5	70.6	−14.1	−20.0	−1.2	−17.7
1947–1948	III, IV, V, VI	76.6	92.4	84.5	15.8	18.7	−21.2	−37.7
1948–1949	II, III, IV, V, VI	93.4	111.7	102.6	18.3	17.8	−2.5	−19.0
1949–1950	II, III, IV, V, VI	111.7	99.8	105.8	−11.9	−11.2	15.3	−1.2
1950–1951	II, III, IV, V, VI	99.8	87.0	93.4	−12.8	−13.7	4.1	−12.4
1951–1952	II, III, IV, V, VI	87.0	105.3	96.2	18.3	19.0	−9.6	−26.1
1952–1953	II, III, IV, V, VI	105.3	113.0	109.2	7.7	7.1	9.4	−7.1
1953–1954	II, III, IV, V	113.0	77.0	95.0	−36.0	−37.9	16.5	0.0
1954–1955	II, III, IV	59.6	66.3	63.0	6.7	10.6	−21.4	−37.9
1955–1956	II, III	11.9	21.0	16.5	9.1	55.2	−10.8	−27.3
1956–1957	II	1.0	8.2	4.6	7.2	156.5	44.4	27.9
1957							200.9	184.4
West Thumb dock:								
1944–1945	VI	4.0	2.2	3.1	−1.8	−58.1	0.0	54.3
1945–1946	V, VI	32.6	24.9	28.8	−7.7	−26.7	−58.1	−3.8
1946–1947	IV, V, VI	49.7	67.5	58.6	17.8	30.4	−84.8	−30.5
1947–1948	III, IV, V, VI	101.7	93.0	97.4	−8.7	−8.7	−54.4	−0.1
1948–1949	II, III, IV, V, VI	99.4	99.3	99.4	−0.1	−0.1	−63.1	−8.8
1949–1950	II, III, IV, V	96.8	95.9	96.4	−0.9	−0.9	−63.2	−8.9
1950–1951	II, III, IV, V	95.9	92.8	94.4	−3.1	−3.3	−64.1	−9.8
1951–1952	II, III, IV, V, VI	94.9	122.4	108.7	27.5	25.3	−67.4	−13.1
1952–1953	II, III, IV, V	120.8	119.3	120.0	−1.5	−1.3	−42.1	12.2
1953–1954	III, IV, V	112.5	72.4	92.5	−40.1	−43.4	−43.4	10.9
1954–1955	III, IV	62.0	81.2	71.6	19.2	26.8	−86.8	−32.5
1955–1956	III	13.2	20.2	16.7	7.0	41.9	−60.0	−5.1
1956							−18.1	36.2

RELATIONS OF AGE AND GROWTH TO FISHING PRESSURE

Fishing *vs.* normal variations

Catches in the Yellowstone Lake fishery increased steadily from 1950 to 1959. Estimated catch for the lake rose almost 100 percent in the 10-year period. Total catch estimates by year were as follows:[3]

1950	200, 015
1951	208, 255
1952	245, 277
1953	195, 873
1954	215, 933
1955	286, 056
1956	290, 221
1957	301, 155
1958	349, 027
1959	393, 467

This section discusses the effect of increased fishing pressure on age and growth of the population.

Mean age provided the most accurate measure of changes in the age composition. Gulland (1955) examined the effects of errors of plus or minus 1 year in aging plaice. He reported that the general effect is to reduce the apparent differences between year classes. The age distribution is leveled out, but the same general shape is retained. The apparent average age is unaltered even if 50 percent of the readings are in error by 1 year. Gulland studied a fish population with a longer lifespan than the Yellowstone Lake cutthroat, but the principle should hold true though to a lesser degree. Use of mean age should minimize the effect of possible errors in age determination of cutthroat due to normal and retarded scales.

Changes in growth rate may be a rather insensitive index of overfishing in Yellowstone Lake. Mottley (1932) stated that overfishing which results in lake depletion is indicated by an increased size for age, among other things. This criterion was only dependable, however, where there was no competition by other species to take up the excess food supply. The longnose sucker (*Catastomus catastomus*) competes directly with the cutthroat for food in Yellowstone Lake.[4] A decrease in density of cutthroat due to heavy fishing pressure may not be reflected by an equally large increase in growth rate if suckers take much of the excess food produced. Data to determine changes in relative abundance and growth rate of the longnose sucker were not collected in previous years, so this possibility could not be explored.

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[3] Figures before 1955 from Cope, 1957a.

Variations in mean annual temperatures were considered another possible factor affecting growth rate. Growth rate can be expected to increase in warmer years and decrease in cold years. A relation has been illustrated between air temperatures and surface water temperatures of a lake (McCombie, 1955); hence, air temperatures were used to indicate warm and cold growing seasons for Yellowstone Lake. Data were obtained from the U.S. Weather Bureau on mean monthly air temperatures at Yellowstone Lake from 1946 to 1958. The period from May to October was considered to be the maximum summer growing season for the comparison. Summation of mean monthly temperatures showed the same fluctuations as percentage deviations from the monthly mean, so the former were used. Calendar years 1948 and 1956 were the warmest during the period and 1950 and 1951 were the coldest (fig. 8). Warm and cold years seemed to have little relation to good and bad years of growth. Correlation coefficients of air temperature and growth rates of fish from combined Fishing Bridge and West Thumb samples were as follows: All ages, 0.21; age groups IV to VI, 0.40; age groups II and III, —0.46. None of the correlations were significant at the 0.05-percent confidence level. Annual fluctuations in growth rate at all ages appeared to be independent of fluctuations in summer air temperatures.

The presence of strong and weak year classes explained some of the observed fluctuations in growth rate and mean age. Significant decreases occurred from 1951 to 1952 in growth rate, mean age, and mean length at capture of creel samples from Fishing Bridge and West Thumb docks (fig. 9). In 1952 the strong 1949 year class (age III) was abundant in the catch. Cope (1953 and 1957a) suggested that the decrease in mean length at capture of fish from 1950 to 1952 (table 1) was the result of increased fishing pressure during that period, and that an increase in mean length in 1953 was the result of weak brood classes of 1950 and 1951. Information now available on growth rate and age composition suggests that the condition resulted from a normal fluctuation in year-class strength. A dominant 1949 year class followed by two weak year classes produced a downward trend in mean age and length in 1952, with an upswing in 1953 as the fish grew older. The decrease in growth rate in 1952 would also be explained if the 1949 year class were sufficiently abundant to cause an increase in population density. Data from Arnica Creek spawning runs support this premise; the 1949 year class from Arnica Creek was the slowest-growing year class produced in the period 1945 to 1956. It was also the most abundant year class during that period.

Growth and mean age for 1954 were nearer the conditions one would expect as a result of a large catch. In both the West Thumb and Fishing Bridge areas, relative growth rate increased and mean age dropped somewhat. Weak 1950 and 1951 year classes could have caused the drop in mean age, but age group V of the dominant 1949 year class should have

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[4] Food habits of the longnose sucker and cutthroat trout in Yellowstone Lake, by Norman G. Benson. Unpublished report, Rocky Mountain Sport Fishery Investigations, Logan, Utah, 1958.

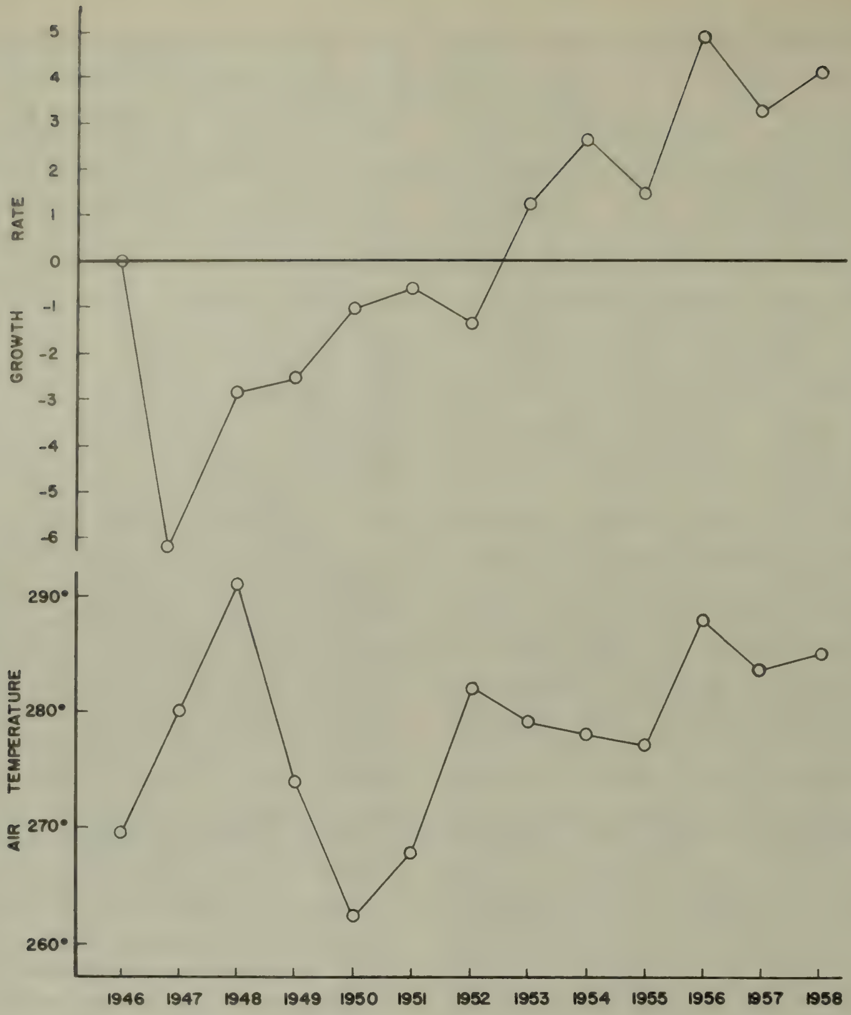


FIGURE 8.—Combined percentage deviations in growth rate of West Thumb and Fishing Bridge creel samples and summation of mean monthly air temperatures (°F.) from May through October at Yellowstone Lake, 1946 to 1958.

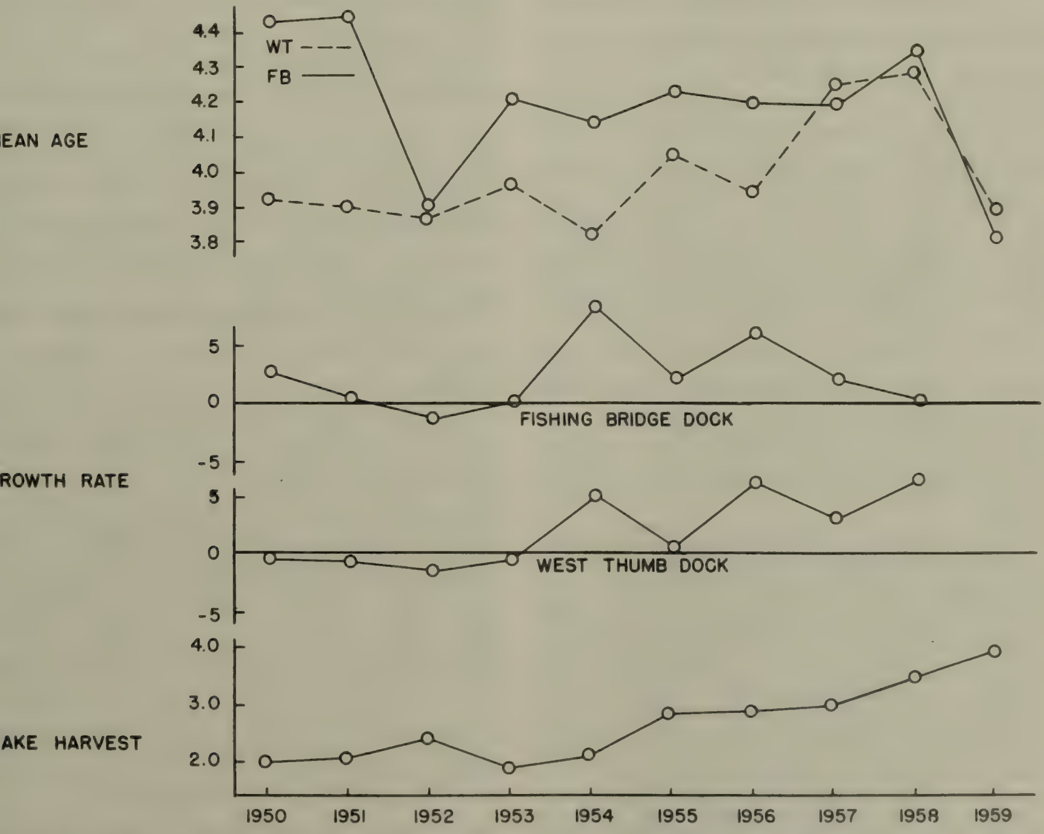
maintained population density. Heavy fishing pressure resulting in a large catch would produce both changes.

Largest deviations from average occurred from 1957 to 1959 in the West Thumb samples. Mean age increased in 1957 and 1958 to a high of 4.28 years followed by a drop to 3.88 years in 1959. Growth rate reached a new high in the period 1956 to 1958. Fishing Bridge samples did not closely follow this trend, except for above average growth rate during the period and a large drop in mean age to a new low of 3.86 years in 1959. If the strong 1953 year class caused the upswing in mean age at West Thumb in 1957 and 1958, its abundance was insufficient to cause a decrease in growth rate in those years. This condition strongly suggests that population density was higher in 1952 than in 1957 and 1958. The decrease in mean age in 1959 appears somewhat ominous. It is suspected that the decrease was not due to strong 1956 and 1957 year classes entering the fishery, but to heavy fishing which delved deeply into these younger age groups. A weakness in the methods used for determining year-class strength is that variations in fishing pressure can give false indications of strong and weak year classes. Hence, the actual strength of the 1956 and 1957 year classes is unknown.

General trends disregarding short-term fluctuations, appear to be of most value in measuring effects of the catch on growth rate. The data indicate that the cyclic variations in mean age and in growth rate were connected with the occurrence and absence of dominant year classes. It was necessary to remove these minor luctuations to determine trends which could be attributed to changes in the catch. A clearer picture of the trend in growth rate is obtained by taking the average of each three consecutive percentage deviations. If the combined percentage deviations in growth rate for West Thumb and Fishing Bridge from table 5 are smoothed twice by moving averages of threes, an upward trend in growth rate becomes apparent:

1946	−3. 07
1947	−3. 34
1948	−3. 00
1949	−2. 46
1950	−1. 49
1951	−0. 87
1952	−0. 12
1953	0. 79
1954	1. 85
1955	2. 61
1956	3. 36
1957	3. 60
1958	3. 84

From 1947 to 1958 a gradual but distinct increase in growth rate occurred. Only



IGURE 9.—Mean age of catch, relative growth rate of collections from Fishing Bridge and West Thumb docks, and annual harvest from Yellowstone Lake. Growth rate expressed as mean percentage deviation from 1946 to 1958, mean age in years, harvest in 100,000 fish.

those age groups fully recruited to the fishery showed this increase in growth rate, and since the increase coincided with a period of increasing catch it was concluded that fishing pressure caused the change.

Northern lake area *vs.* southern lake area

Changes which have occurred in the population are perhaps more evident when comparisons are made between spawning populations in the north and south ends of the lake. Cope (1953) estimated that 95 percent of the fishing pressure was expended on the northern end of the lake. That percentage has undoubtedly decreased slightly in recent years owing to the large number of private trailer boats and cruisers on the lake; but the northern end of the lake, being highly accessible, still receives most of the fishing pressure. Another factor also protects the southern spawning runs. Greatest fishing intensity on the lake occurs during late June and early July (Cope, 1957a). Spawning in Grouse and Chipmunk Creeks reaches a peak at the same time (Cope, 1957b). Hence, most of the catchable-sized fish of these two streams do not complete spawning and move northward until fishing pressure has declined. Harvest of the southern populations is correspondingly less, and some differences in the populations from the north and south ends of the lake are probably the result of this uneven exploitation.

Several comparisons were made between spawning populations from Grouse and Chipmunk Creeks and from Arnica, Pelican, and Clear Creeks. The significantly larger mean lengths at capture of fish from Grouse and Chipmunk as contrasted with mean lengths of fish from northern streams was mentioned previously (table 1). The present study shows that this length differential was the result of differences in age composition of the runs. Spawning runs in Chipmunk and Grouse Creeks contained larger percentages of old fish. Ball and Cope (1961) reported that average spawner survival is lower in Grouse and Chipmunk Creeks than in the three northern streams listed above. Thus, one would expect mean age of runs in these two streams to be lower, as fewer old fish survive the rigors of spawning. A larger percentage of old fish is present which indicates that the low mean age of the northern spawning runs is the result of the larger catch in that area.

A difference was noticed also in the percentage of spawners with normal scales between north and south areas (table A–1, appendix B). An analysis of variance was made of differences in the percentage of age IV spawners with normal scales in the 1953 to 1955 collections. There was a significant difference at the 0.05-percent level (*F* value, 34.11) in percentages of fish with normal scales among five spawning runs. Combined samples from Arnica and Pelican were significantly different from Chipmunk and Grouse Creek samples with an *F* of 42.97. Differences between Chipmunk and Grouse Creeks were very small, as an *F* of 0.40 was not significant at the same probability level.

Variations in production of fish with normal scales may be the result of differences in stream environment, time of spawning, or length of time fry remain in the stream. Hence, comparison of percentage differences in the number of fish with normal scales from one year of age to the next provides a more accurate comparison. Percentage decreases in normal scales from age IV to age V in the 1953 to 1955 samples were: Arnica, 39.1; Pelican, 38.2; Clear, 33.3; Chipmunk, 27.5; and Grouse, 17.5. Normal fish from the northern spawning runs obviously had a higher mortality between age IV and V than did fish from Grouse and Chipmunk runs. This observation was supported by high calculated lengths of older fish in the southern runs, and also explains some of the difference in degree of Lee's phenomenon in samples from the northern and southern areas (appendix B).

Discussion of angling effects

Increase in catch of fish on Yellowstone Lake from 1950 to 1959 caused a decline in the percentage of fish belonging to age groups VI and VII, especially in the northern end of the lake. Fish in these two age groups were apparently never very abundant in the population. The amount of reduction in mean age of the catch due to fishing pressure has not been determined. Mean age varied more with year-class strength than with fishing pressure, although the drop in mean age to a new low in 1959 is attributed to the exceedingly heavy catch in that year. Mean age of the population was undoubtedly reduced somewhat in proportion to size of the catch, but not enough to dampen out fluctuations caused by the occurrence of strong and weak year classes.

The decrease in population density resulting from the large catch caused an increase in growth rate of mature cutthroat even with a competing species of fish present. This increased growth rate balanced out loss of older fish from the population so that mean length of the catch has not declined.

The observed increase in growth rate and decrease in percentage of fish in the older age groups indicate that production in the lake has been more efficient, and the 1959 catch may be close to a maximum equilibrium yield. The removal of large old fish often allows an increase in numbers of fish of medium size, and the total number of fish available to the fishery is increased. The improvement is associated with an increase in growth rate. Hence, increase in growth rate of Yellowstone Lake cutthroat is probably a healthy condition as mean size of fish in the catch has not decreased. An excessive catch will be indicated, however, if growth rate remains high but mean size and age of the catch decrease more than can be attributed to variations in year-class strength. The present rate of increase in fishing pressure and the changes in age and growth which have taken place suggest that this condition may occur within the next few years.[5]

SUMMARY

Scale collections from 1948 to 1959 provide considerable information on the growth rate and age composition of the cutthroat population in Yellowstone Lake. Growth rate of combined samples of the catch was slow from 1947 to 1952 and most rapid in 1954, 1956, and 1958. Rate of growth increased gradually from 1947 to 1958 when annual fluctuations were smoothed out. There was no correlation between annual fluctuations in mean summer air temperature and growth rate.

Females are predominant both in the spawning runs and in the catch up to age V or VI; males then become predominant.

Fish in the spawning runs range from 3 to 7 years of age. Fish of age groups IV and V composed 77 to 98 percent of the runs examined from 1951 to 1959. Age group IV was usually predominant. Mean age of fish from Chipmunk and Grouse Creeks is much higher than that of fish from spawning runs in the northern end of the lake. Age composition of the catch is similar to the spawning runs with the exception of larger percentages of age III fish and the inclusion of some age II fish in the catch. A general decrease occurred in the percentage of age VI and age VII fish in the catch during the period from 1950 to 1959. Mean age of the catch fluctuated independently of fishing pressure.

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[5] Recent analysis of 1960 data indicates that maximum equilibrium yield was surpassed in 1959. It was not evident that the 1959 catch was excessively high until the 1960 data on growth rate, mean lengths, and age composition of the catch and spawning runs were available.

Year class strength varied in different spawning streams, although 1949 produced a strong year class in most areas. Samples from the catch at Fishing Bridge dock indicated that strong year classes were produced in 1945, 1949, and from 1953 to 1957; weak year classes were produced in 1947 and 1951. West Thumb samples had strong year classes in 1944, 1947, and from 1952 to 1956; 1946 and 1948 to 1951 produced weak year classes.

The general increase in growth rate and a decrease in percentage of older age groups are assumed to be the result of the increase in catch of fish.

The observed changes suggest that more efficient production has occurred and that maximum equilibrium yield is near. If the catch continues to increase at the present rate, it may become excessive within the next few years.

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Appendix A—BODY SCALE RELATION

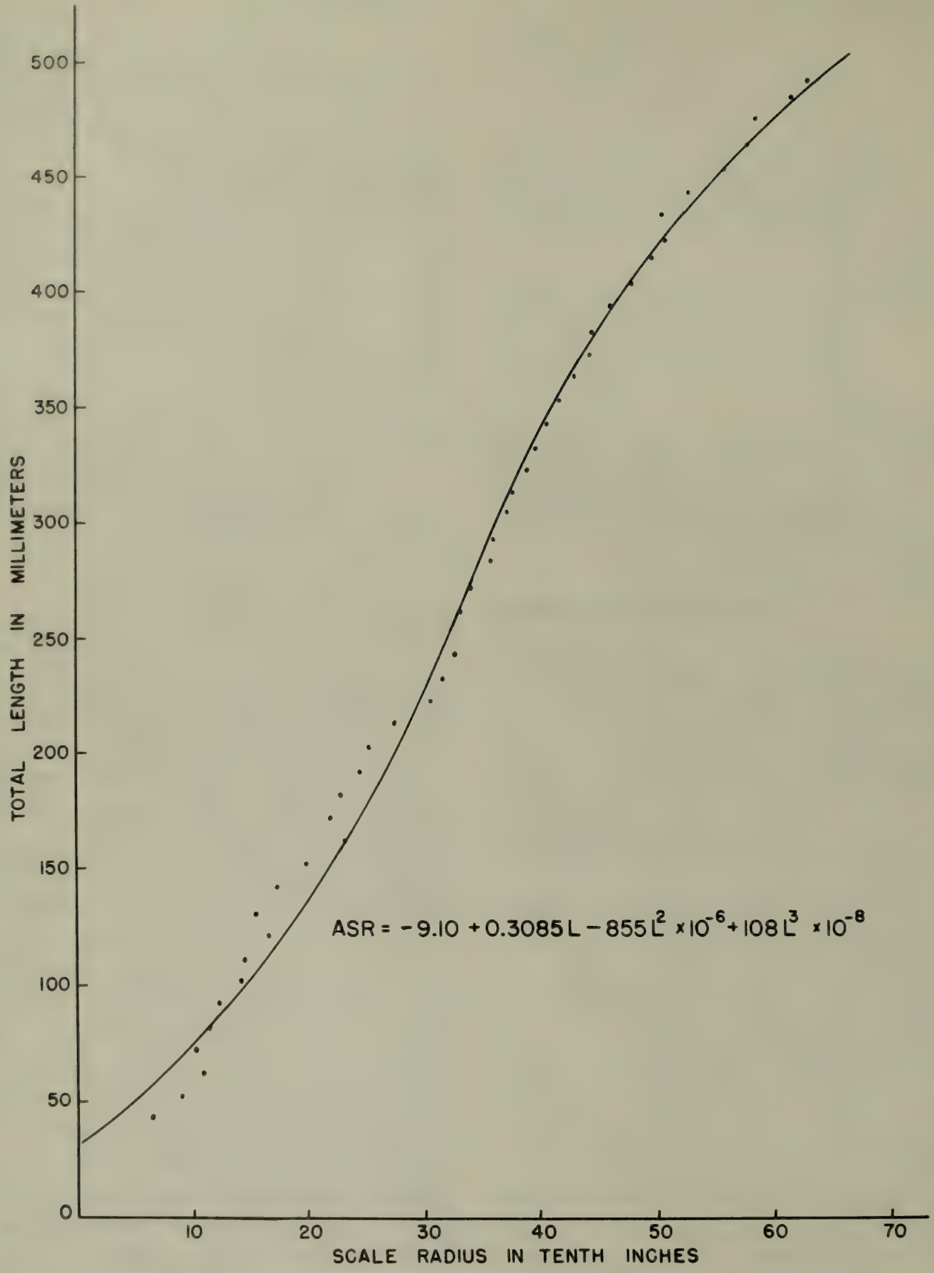
Workers studying cutthroat trout have reported various types of body-scale relations. Fleener (1952) obtained an exponential relation for cutthroat of Logan River, Utah. Irving (1956) found that a second-degree polynomial best described his data for Henrys Lake cutthroat. Willoughby (1951) and Bjornn (1957) used a linear relation for cutthroat of Yellowstone Lake and Priest Lake, respectively. The different expressions of the body-scale relation may be due to limited sample sizes rather than to actual differences in growth of scales in relation to body growth.

Laakso (1956) described the body-scale regression of juvenile cutthroat from Yellowstone Lake as linear and found significant differences in this relation for fish from four areas of the lake. A linear relation probably exists for adult Yellowstone fish within the size range of 300 to 425 millimeters. This size range included approximately 90 percent (8,290 fish) of samples of the catch and spawning runs collected from 1950 to 1956. A test for linearity within the latter size range provided an F value of 1.06 which was not significant at the 0.05-percent level of probability. Thus, a straight line satisfactorily expresses the regression of the anterior scale radius on body length for fish from 300 to 425 millimeters. Analysis of variance also indicated that there was no significant difference in the body-scale relation among fish in this size range from different parts of the lake. An F value of 0.897 was not significant at the 0.05-percent level. Even though some minor differences in the body-scale regression of juvenile fish may be present as suggested by Laakso, the relation is similar for adult fish.

The body-scale regression for fish of all lengths was determined by measurement of 9,668 fish from 40 to 500 millimeters in total length (fig. A–1). The relation for fish of all sizes was best described mathematically as a sigmoid curve expressed by the formula

$$ASR = -9.10 + 0.3085\ L - 855\ L^2 \times 10^{-6} + 108\ L^3 \times 10^{-8},$$

where ASR equals the anterior scale radius



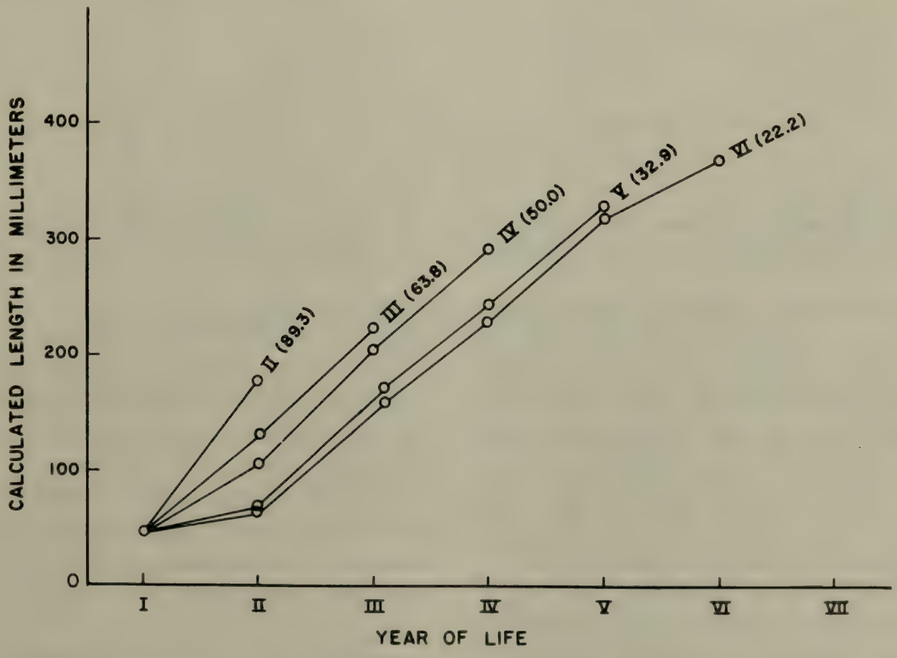
Appendix figure A–1.—Body-scale relation of Yellowstone Lake cutthroat.

in tenths of an inch (×115) and L is body total length in millimeters. This formula does not adequately describe the body-scale relation for fish below 250 millimeters in total length. A regression line drawn freehand to conform closely with the empirical data was used for construction of a nomograph in the manner described by Lowry (1951). The method provided more accurate calculated lengths than those obtained from the theoretical body-scale formula.

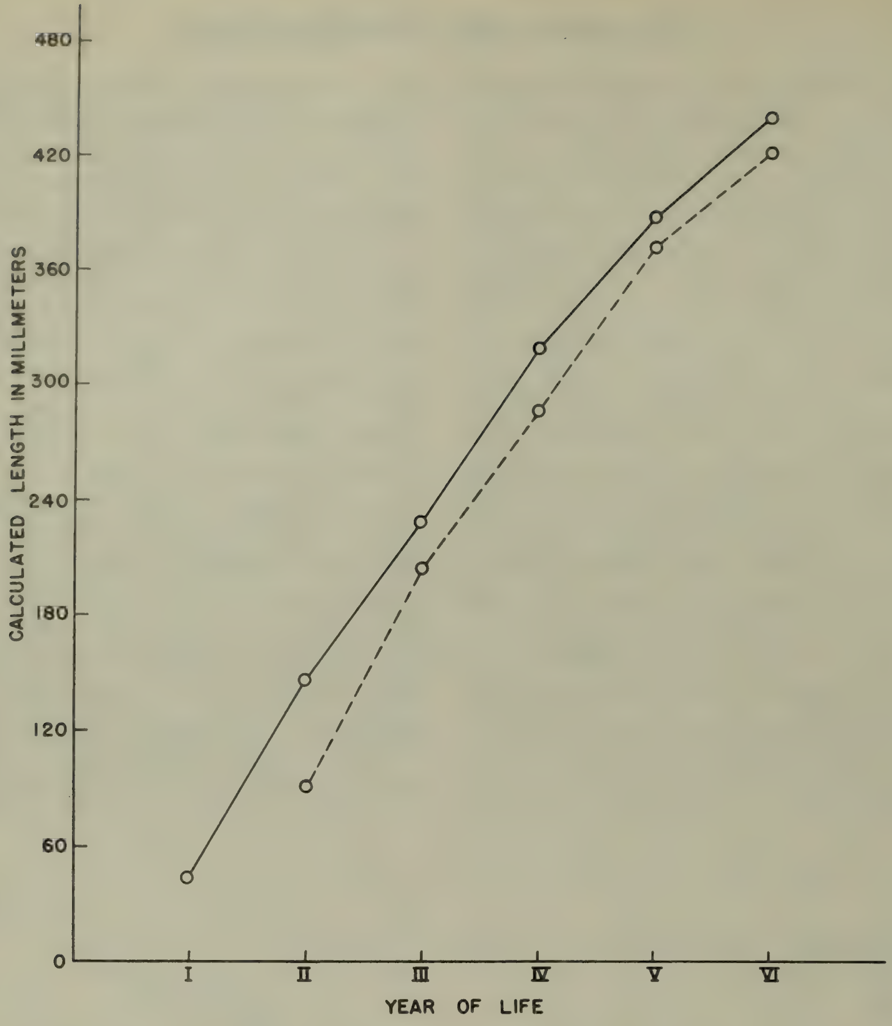
Appendix B—LEE'S PHENOMENON

Lee's phenomenon was evident to such a degree in the samples that correction was necessary to reduce its effect on calculated lengths. The 1954 collection from West Thumb dock illustrates this phenomenon (fig. A–2). Calculated lengths to most ages by older fish were smaller than lengths calculated from the younger fish. Formal *t* tests to determine the degree of differences in calculated lengths were made. Calculated lengths at age II from age III fish of the 1955 collections were compared with lengths at age II calculated from older fish. Differences in calculated lengths were significant at the 0.05-percent level for collections from Arnica, Pelican, and Clear Creeks and from the West Thumb and Fishing Bridge segments of the fishery. Tests of the differences in calculated lengths at age III by age group III fish and lengths calculated at age III by older fish provided values of *t* which were significant at the 0.05-percent level with the exception of Clear, Grouse, and Chipmunk Creek samples. Lee's phenomenon was much less evident in Chipmunk and Grouse Creek collections than in samples taken at the north end of the lake.

Hile (1936) lists the possible causes of Lee's phenomenon in calculated lengths. The presence of normal and retarded scales provided a unique illustration of the causes of Lee's phenomenon in this study. Normal fish were larger on the average than fish with retarded scales of the same age up to age V (fig. A–3). The percentage of these larger fish with normal scales decreased with age in all collections examined (table A–1). This decrease was not due to differences in the percentage of normal fish produced in certain years, as the percentage remained fairly constant for a given area. Percentages of fish of age groups III, IV, and V with normal scales from six year classes of Arnica Creek spawners were



Appendix figure A–2.—Calculated total lengths from combined age groupsof 455 fish collected at West Thumb dock, 1954. Percent of fish in sample with normal scales in parentheses.



Appendix figure A–3.—Growth in length of 697 normal (solid line) and 952 retarded fish (broken line) from combined collections of 1955. Calculated total lengths by summation of mean increments.

not significantly different (table A–2). Analysis of variance among years in the percentage of fish with normal scales provided an *F* value of 2.086, which was not significant at the 0.05-percent level. Hence, the larger (normal) fish of each year class obviously suffered an early mortality, leaving the smaller (retarded) fish to make up the older age groups.

This differential mortality was attributed to two factors. Larger fish are recruited into the spawning runs and into the fishery sooner than smaller fish of the same year class. Spawning mortality is high and few fish survive to spawn a second time (Ball and Cope, 1961). Thus, Lee's phenomenon was caused not only by selective fishing but also by a higher natural mortality among the larger fish of each year class.

Only fish in age group IV and younger were combined for calculating lengths up to age III to compensate for Lee's phenomenon.[1] Inclusion of age IV fish in

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[1] Adjustment for Lee's phenomenon was applied only in tables A–3 and A–4 where calculated lengths at each year of life are presented; all other comparisons were made with unadjusted calculated lengths.

Appendix table A–1.—*Percent of fish with normal scales in each age group from the 1955 collection at Yellowstone Lake*

[Number of fish examined in parentheses]

Area	Percent of fish with normal scales in age group—				
	II	III	IV	V	VI
Arnica	------	50.0 (26)	28.9 (190)	12.4 (89)	0.0 (4)
Pelican	------	59.0 (29)	37.7 (106)	25.6 (82)	17.6 (17)
Clear	------	100.0 (7)	90.1 (101)	80.0 (25)	------
Chipmunk	------	0.0 (3)	67.3 (161)	34.9 (83)	15.8 (14)
Grouse	------	------	56.5 (46)	43.5 (39)	50.0 (6)
West Thumb	80.0 (25)	66.1 (118)	47.6 (145)	22.8 (70)	44.4 (9)
Fishing Bridge	90.0 (9)	45.0 (60)	27.5 (80)	23.6 (123)	7.7 (26)

the computations helped compensate for the fish in age II and III which were the largest of their age group. Calculated lengths for age IV and above were obtained by consecutively adding mean annual increments of combined age groups of older fish to calculated length at age III. This method gave fair agreement between calculated and empirical lengths of each age group (table A–3). A calculated length of 45.2 millimeters for the first year of life agreed well with empirical lengths of age I fish. Laakso and Cope (1956) measured 190 age group I fish captured from May 5 to May 17, 1953. Mean total length of these fingerlings was 47.8 millimeters. They reported that annulus formation is completed before May 5 by most fish under 330 millimeters in length. Calculated lengths at the end of the first year of life in the present study ranged from 43 to 56 millimeters. Greatest differences between calculated and empirical lengths were at ages II and III, but all samples included only the largest fish in these age groups.

Appendix table A–2.—*Percent of fish with normal scales in six year classes from Arnica Creek collections of 1951 to 1958*

[Number of fish examined in parentheses]

Age when examined	Percent of fish with normal scales in year class of—					
	1948	1949	1950	1951	1952	1953
III	35.3 (34)	35.3 (51)	52.1 (23)	47.8 (23)	50.0 (26)	56.3 (16)
IV	19.7 (152)	18.0 (122)	17.5 (74)	28.9 (190)	45.0 (91)	27.5 (138)
V	13.3 (60)	14.8 (94)	12.4 (89)	13.5 (52)	11.6 (129)	17.1 (53)

Appendix table A–3.—*Mean total lengths at capture and calculated total lengths adjusted for Lee's phenomenon from the 1954 Fishing Bridge collection of 229 fish*

[In millimeters]

Year of life	Length at capture		Calculated length
	Mean	Range	
I	--------	----------	45.2
II	280.5	200–305	131.2
III	304.7	260–340	215.9
IV	340.8	315–390	306.0
V	375.3	350–415	380.8
VI	400.9	395–415	432.8

Appendix C—CALCULATED LENGTHS

Calculated lengths adjusted for Lee's phenomenon and mean lengths at capture of combined collections from 1953 to 1955 are presented in table A–4. No difference was determined in calculated lengths of males and of females, so sexes were combined for all comparisons. Calculated lengths of fish from Clear, Chipmunk, and Grouse Creeks were generally larger than lengths of fish from other spawning runs. Range in calculated length among areas was least at the end of the first year of life (5.6 millimeters) and greatest at the end of the second year (39.7 millimeters). Calculated lengths of older age groups were larger than lengths at capture because only the smaller fish of each year class survived to ages VI and VII. Actual differences in calculated lengths between areas were minor, and most of the variation was connected with different percentages of normal fish in the samples; the three areas with supposedly larger fish also

Appendix table A-4.—*Mean calculated total lengths adjusted for Lee's phenomenon of fish from combined collections of 1953 to 1955*

[In millimeters. Mean lengths at capture in parentheses]

Area	Number of fish	Mean calculated length at age—						
		I	II	III	IV	V	VI	VII
Arnica	744	49.7	118.9	218.0	306.0	385.6	437.6	--------
			(169.0)	(301.3)	(342.3)	(370.7)	(421.2)	--------
Pelican	661	44.1	113.3	219.6	306.8	390.3	439.8	481.8
				(306.7)	(344.0)	(382.0)	(427.8)	(440.0)
Clear	471	45.0	144.8	225.3	317.0	399.6	453.5	490.5
				(304.3)	(355.8)	(377.1)	(424.8)	(455.0)
Chipmunk	672	46.0	153.0	227.0	323.7	402.2	454.5	488.5
				(318.7)	(358.0)	(394.5)	(429.6)	(447.3)
Grouse	345	47.9	138.0	226.2	327.9	395.4	440.1	476.1
				(326.9)	(363.5)	(396.0)	(422.2)	(445.0)
West Thumb	1,180	46.5	121.0	216.0	297.4	380.7	430.2	477.2
			(283.0)	(297.0)	(349.7)	(364.5)	(390.5)	(402.5)
Fishing Bridge	984	45.0	131.3	243.0	320.8	406.3	457.1	497.1
			(268.6)	(298.0)	(342.6)	(374.6)	(406.3)	(423.0)
Weighted mean length	5,057	46.2	129.3	225.0	311.8	393.2	443.8	485.4

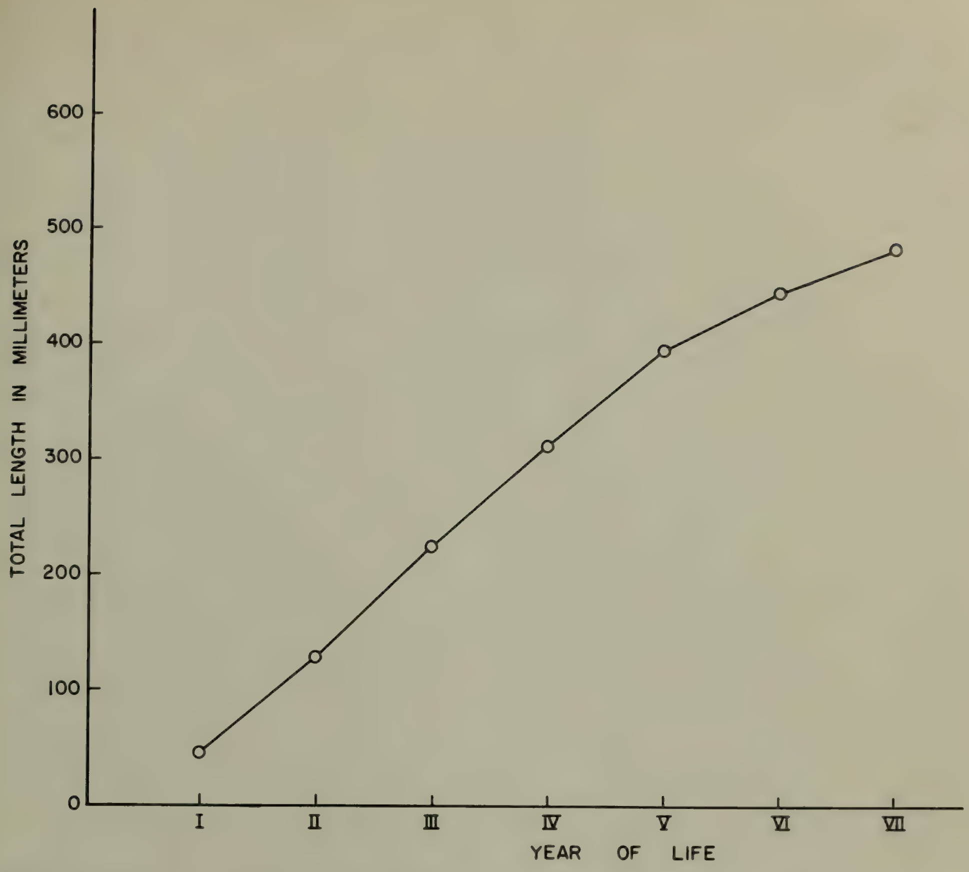
had the highest percentages of normal fish in the samples.

A growth curve derived from mean lengths does not necessarily represent the growth pattern of an individual fish, but the calculated lengths presented in table A-4 do indicate the theoretical pattern of growth of Yellowstone Lake cutthroat. The growth curve for combined collections of 1953 to 1955 from all areas of the lake revealed that mean growth was most rapid between ages II and IV (fig. A-4). Growth rate decreased slightly between ages IV and V; after age V growth slowed rapidly.

Appendix D—GROWTH COMPENSATION

Comparison of growth increments suggested that the early disadvantage in size of fish with retarded scales was not completely compensated in later life. Calculated lengths by mean increment summation were higher for normal fish than for retarded fish at all ages. Figure A-3 illustrates this length difference. Comparison of increments of length in millimeters attained at each age by normal fish and by retarded fish collected in 1955 from all areas of the lake were as follows: First year, 44.3 and——; second year, 101.7 and 90.6; third year, 81.9 and 113.2; fourth year, 89.9 and 82.7; fifth year, 68.6 and 85.0; sixth year, 53.0 and 49.0. The retarded fish had a faster average rate of growth after the first year of life. Differences in calculated lengths between the two groups decreased with age, but individual fish which were undersized at the end of the first year of life did not grow sufficiently fast to catch up with the normal fish. Van Oosten (1929) described a similar condition for the lake herring of Lake Huron, where the small yearlings grew more rapidly than large yearlings.

Another factor which must be considered in analyzing growth compensation in the Yellowstone Lake population is the high mortality of normal fish mentioned in the section on Lee's phenomenon. Normal fish were recruited into the fishery and the spawning runs at a younger age than retarded fish and were thus removed from the population at a young age. The above analysis of growth compensation using length increments does not consider this mortality differential. If only the normal and retarded fish which survive are used for comparison, it can be shown that a distinct growth compensation does occur. Calculating lengths by combined age groups instead of by mean increment summation illustrates this condition. Calculated lengths in millimeters of the 1955 sample of 697



Appendix figure A–4.—Growth curve of Yellowstone Lake cutthroat based on calculated lengths of 5,057 fish collected from 1953 to 1955.

normal fish and 952 retarded fish are as follows: First year, 44.3 and——; second year, 146.0 and 90.6; third year, 226.6 and 203.8; fourth year, 309.7 and 284.0; fifth year, 360.2 and 348.3; sixth year, 387.0 and 405.0. By this method surviving normal fish were larger than retarded fish only up to the fifth year of life. Apparently the normally scaled fish still living at age V were the slowest-growing individuals in that group, as they had been overtaken by the rapid-growing retarded fish.

Another explanation is that spawning at a younger age caused a decline in rate of growth of the normal fish so that the slower-maturing retarded fish were able to catch up. Ball and Cope (1961) described the deleterious effects of spawning on Yellowstone Lake cutthroat. Calhoun (1944) reported that cutthroat in Upper Blue Lake, Calif., failed to grow after spawning. Yellowstone Lake fish which survive spawning do continue to grow, although perhaps at a slower rate. In calculated lengths of age V fish, the difference between normal and retarded fish was negligible (11.9 millimeters). Mean calculated lengths of the retarded fish had even surpassed mean length of the few remaining normal fish by the sixth year of life. Hence, where only surviving fish are considered, growth compensation between retarded and normal fish is completed by the end of the fifth year of life.

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MORTALITY STUDIES ON CUTTHROAT TROUT IN YELLOWSTONE LAKE

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 55

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MORTALITY STUDIES ON CUTTHROAT TROUT IN YELLOWSTONE LAKE

Orville P. Ball and
Oliver B. Cope
Fishery Research Biologists

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ABSTRACT

In a study of the Yellowstone cutthroat trout, *Salmo clarki lewisi,* by the U.S. Fish and Wildlife Service, effects of environment on mortality of eggs, immature fish, spawners, and postspawners were measured for various components of the population in Yellowstone Lake (Wyoming). Five methods for estimating mortality of adults on spawning runs are described, with counting and tagging as the principal procedures. Of the total number of eggs deposited in the gravel, 60 to 75 percent died before hatching, and 99.6 percent had died by the time the fingerlings entered Yellowstone Lake. Among adults on spawning runs, mortality was 48.1 percent. Sex ratios on spawning runs were uneven, averaging 1.47 females to 1 male. Very few trout spawned a second time; of those that did, more spawned after a 2-year interval than after 1 year.

In the Arnica Creek runs, 48.6 percent died in the stream, 40.2 percent died later in the lake of natural causes, 7.6 percent were taken by fishermen, and 3.6 percent were alive 2 years later. The white pelican is a serious predator on cutthroat trout in Yellowstone Lake. From 1949 to 1953, fishermen caught 11.6 percent of the catchable trout available to them. Migrations of adult fish in Yellowstone Lake were traced through tagging.

MORTALITY STUDIES ON CUTTHROAT TROUT IN YELLOWSTONE LAKE

By Orville P. Ball and Oliver B. Cope
Fishery Research Biologists
Rocky Mountain Sport Fishery Investigations
Logan, Utah

The U.S. Fish and Wildlife Service has been engaged in a full-scale study of the Yellowstone cutthroat trout, *Salmo clarki lewisi*, in Yellowstone Lake (Wyoming) for several years. The lake lies within Yellowstone National Park and has become increasingly prominent among the recreational features of the park, especially since World War II. Large increases in fishing pressure and noticeable changes in the fishery developed by 1950, and the National Park Service sought advice from the Fish and Wildlife Service regarding reasonable management methods for this fishery resource.

The problem, one of population dynamics, in Yellowstone Lake was to find the numbers of cutthroat trout, and their sizes, that could safely be taken from these waters. The approach to the problem resolved itself into studies of mortalities and survivals, and still continues in this direction. Studies on the various mortalities operating on the population of cutthroat have resulted in an abundance of data on this species in Yellowstone Lake and tributary waters. Since the cutthroat has received relatively little attention in the past by students of trout biology, the present paper brings together mortality information gathered from the Yellowstone study so that the biology of this important sport fish of western North America can be better understood. Emphasis is placed on mortalities of trout eggs in the gravel, of alevins, of fry and fingerlings in streams, and of adults in spawning runs in streams, and on fishing and natural mortality of adults in Yellowstone Lake after their return to the lake from spawning in the streams.

Information pertaining to the fish and fisheries of Yellowstone Lake dates from the survey of David Starr Jordan in 1889 (Jordan, 1891). Several years later a more comprehensive description of the fish species of the Yellowstone area was published (Evermann, 1893). Some delay was experienced before additional literature appeared concerning the fishes of the park, particularly Yellowstone Lake (Kendall, 1915; Smith and Kendall, 1921; Simon, 1953).

A parasitic tapeworm common among the trout of Yellowstone Lake was first described by Leidy (Hayden, 1871). Hayden himself remarked in this report (p. 97) "We are able to discover but one species of fish in the lake (Yellowstone): and that was trout, weighing from two to four pounds each. Most of them are infested with a peculiar intestinal worm which has been described by Dr. Leidy in a subsequent portion of this report, under the name *Dibothrium cordiceps.*" Later

---

Orville P. Ball is now Fish and Game Specialist with the City of San Diego, Calif. Oliver B. Cope is now Chief, Fish-Pesticide Research Laboratory, U.S. Fish and Wildlife Service, Denver, Colo.

additional material concerning the anatomy and life history of *D. cordiceps* was compiled (Linton, 1891a; Linton, 1891b; Woodbury, 1934;[2] Bangham, 1951). An important study dealing with this tapeworm and its relation to one of its hosts, the white pelican (*Pelecanus erythrorhynchus*), and which included a quantitative evaluation of predation by pelicans on the trout of Yellowstone Lake was completed by Ward (1922 [3]). Several articles have been devoted to the white pelican not only because of its predator-prey relation to the cutthroat trout, but also because of the very important aesthetic niche the bird fills in the park (Grinnell, 1876; Skinner, 1925; Thompson, 1932).

Within recent years several papers on changes in the fishery at Yellowstone Lake have been published (Moore, Cope, and Beckwith, 1952; Cope, 1957). The biology of the cutthroat of Yellowstone Lake has been studied by Brown and Bailey (1952), Laakso (1955, 1956), Laakso and Cope (1956), Cope (1953a, 1953b, 1956, 1957a, 1957b), and Ball (1955).

The authors are grateful to the National Park Service for cooperation throughout this study. The Yellowstone Park Company extended the assistance of its Boat Division in the compilation of creel-census data and reporting tagged fish caught. The late William A. Dunn, Superintendent, U.S. Fishery Station at Yellowstone Lake, gave considerable assistance to our staff. Many field biologists should be thanked for their contributions to the tagging program. Several research biologists including Martin Laakso, R. A. Fredin, Theodore Widrig, W. F. Carbine, Ross V. Bulkley, and Drs. Ralph Hile James W. Moffett, and Norman G. Benson, have critically read the manuscript.

BACKGROUND MATERIAL

Yellowstone Lake and tributaries

Weather conditions in the Yellowstone Lake area range from severe winters with abundant snowfall and subzero temperatures to mild, pleasant summers. Winter usually begins in September or October and extends through May. Snow cover on the level may reach a depth of more than 6 feet. The lake usually freezes over in the fall and remains ice-covered until the last of May or early June. These rigorous physical conditions influence the seasonal productivity of the lake and the biology of the fish.

Yellowstone Lake, elevation 7,500 feet, has a surface area of about 90,000 acres and a shoreline of nearly 100 miles (fig. 1). Maximum recorded depth is 325 feet. The lake has 14 major and 26 minor tributary streams.

The principal tributaries used for spawning are Pelican, Clear, and Cub Creeks in the northern section, Columbine and Beaverdam Creeks and the upper Yellowstone River in the Southeast Arm, Chipmunk and Grouse Creeks in the South Arm, and Arnica and Solution Creeks in West Thumb. The lower Yellowstone River drains from the north end of the lake. Fifteen miles below the outlet of the lake is a waterfall which is a fish barrier.

The stream studies in this paper are based on five streams, which are briefly described here.

Arnica Creek drains approximately 20 square miles and is 5.8 miles long.[4] It is the smallest tributary on which extensive tagging took place. The water flow at the trap during the summer, while normally 5 to 7 cubic feet per second, has surpassed 20 c.f.s. during spring floods. Warm springs are largely the cause of moderately high stream temperatures.

Pelican Creek, with almost 100 miles of stream, is the largest tributary at which

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[2] Woodbury, L. A., Parasites and diseases of the trout of Yellowstone Lake (*Salmo lewisi*), Master of Science thesis, University of Utah, 1934, 60 pages.

[3] Ward, H. B., The pelicans of Yellowstone Lake. Manuscript prepared for U.S. Bureau of Fisheries in cooperation with the U.S. National Park Service, 1922, 20 pages.

[4] Welsh, James P. A population study of Yellowstone black-spotted trout (*Salmo clarkii lewisi* Girard). Ph. D. dissertation, Stanford University, 1952. 180 pp.

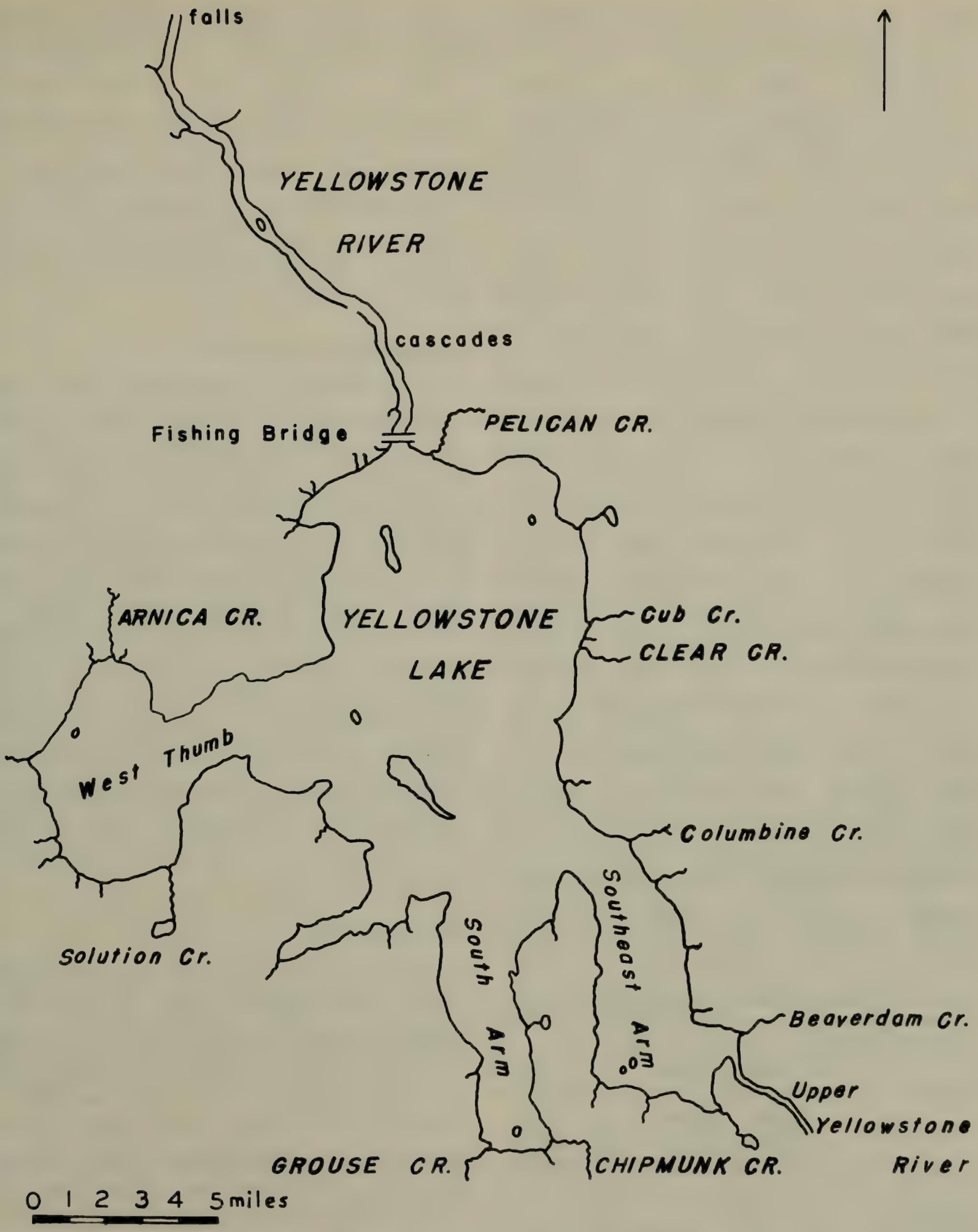

FIGURE 1.—Yellowstone Lake and Yellowstone River, with principal tributaries.

tagging occurred. The drainage area is about 60 square miles. The water flow was measured at 37 c.f.s. during low water in the fall of 1953, but has exceeded 1,000 c.f.s. during June in some years. Numerous warm springs and several geyser basins in this watershed generally restrict spawning to areas least under the influence of warm mineralized water.

Clear Creek on the east shore of Yellowstone Lake includes a total of approximately 24 stream miles. The water flow at the trap was 20 c.f.s. in the middle of August, 1953. The stream has few warm springs and aquatic conditions appeared to be most ideal throughout the summer.

Spawning gravel is well distributed throughout the watercourse.

Chipmunk Creek has almost 22 miles of stream accessible to fish. Midsummer water flow at the trap has usually averaged around 26 c.f.s. Most of the spawning beds are in the lowest 9 miles of the stream and in its several tributaries.

Grouse Creek and its tributaries, draining about 12 square miles, is more than 9 miles long. By early fall, the water flow near the mouth was normally about 13 c.f.s. Only the lower 3 miles are utilized to any extent for spawning. Hot mineral spring activity which influences Arnica and Pelican Creeks so markedly is lacking in the tributaries to the South Arm.

The weekly averages of daily maximum temperatures from thermograph records are available for Arnica, Pelican, Grouse, and Chipmunk Creeks. Table 1 shows temperature patterns at trap locations, expressed as mean maximum weekly temperatures. These data describe the historical high stream temperature for each week during the spring and summer spawning period. Arnica Creek had the highest average seasonal temperature of the streams studied, and warmed up most rapidly in the spring. However, Pelican Creek attained higher average weekly temperatures. Water temperatures in Grouse and Chipmunk Creeks were lower than in Arnica and Pelican Creeks. Records for 1953 and 1954 (not given in table) indicate that temperatures in Clear Creek were similar to those of Grouse and Chipmunk Creeks. Marked diurnal fluctuations in water temperatures are characteristic of the tributaries to Yellowstone Lake.

TABLE 1.—*Mean maximum weekly temperatures from thermograph records in four streams tributary to Yellowstone Lake*

[In degrees Fahrenheit]

Time of season	Arnica Creek 1950–53	Pelican Creek 1950–53	Grouse Creek 1951	Chipmunk Creek 1950–51
May 4–10	43	47	----------	----------
May 11–17	48	43	----------	----------
May 18–24	49	45	----------	----------
May 25–31	53	50	----------	39
June 1–7	53	49	46	40
June 8–14	57	50	44	46
June 15–21	60	51	50	53
June 22–28	60	52	49	51
June 29–July 5	65	59	54	56
July 6–12	64	63	54	55
July 13–19	67	65	60	62
July 20–26	66	68	61	62
July 27–Aug. 2	65	68	61	61
Aug. 3–9	63	65	58	60
Aug. 10–16	64	67	55	61
Aug. 17–23	65	67	----------	----------
Aug. 24–30	62	64	----------	----------
Aug. 31–Sept. 6	60	63	----------	----------
Sept. 7–13	61	57	----------	----------
Seasonal mean	59.2	57.5	54.0	53.8

Fish hatchery operations

A fish hatchery at Yellowstone Lake was operated from the early 1930's to 1953. The hatchery maintained fish traps near the mouths of Pelican, Cub, Clear, Chipmunk, and Grouse Creeks, and obtained eggs from wild spawners as they ascended the streams in late spring and early summer. Eggs were taken from some fish in each stream, and the rest of the fish were allowed to ascend the streams to reproduce naturally. Most of the eyed eggs from the Yellowstone hatchery were shipped out of the park to State and Federal fish hatcheries for rearing and planting in other areas. The remainder was returned to Yellowstone Lake and other waters within the park.

The fish fauna

The cutthroat trout is the only game fish in Yellowstone Lake. It is the most abundant fish in this water, and ranges widely. This trout spawns in most of the streams tributary to the lake, inhabits all sections of the lake, and ranges downstream in the Yellowstone River. Below the falls in Yellowstone River the cutthroat is found with other species of trout.

The longnose sucker, *Catostomus catostomus* (Forster), is found in Yellowstone Lake and is occasionally taken by anglers in the northeast section of the lake, in shallow water. Spawning runs enter Pelican Creek regularly, and records indicate that other streams are occasionally used by this species.

Two cyprinids, the longnose dace, *Rhinichthys cataractae* (Valenciennes), and the smallfin redside shiner, *Richardsonius balteatus hydrophlox* (Cope), also occur in this drainage.

Spawning runs

Spawning of cutthroat trout takes place during late spring and early summer, somewhat later in the year than the spawning of most cutthroat populations in surrounding Rocky Mountain waters. After ascending from the lake, adults usually spend 2 or 3 weeks in the stream. During this time they participate in the upstream migration, nest building, spawning, and the downstream migration. Adults that survive spawning move back into the lake. Yolk-sac fry emerge from the gravel in the summer or early fall. Most fry begin a downstream movement immediately and spend their first winter in the lake. Others remain in the streams and first enter the lake as fingerlings in the following summer. A few may continue stream residence still another year, but no evidence suggests that trout mature in the tributary streams rather than in the lake or lower Yellowstone River. These trout become sexually mature for the first time as 3- or 4-year-old fish.

In the present paper the term "yolk-sac fry" is used for immature fish whose yolk-sac has not yet been absorbed; "fry" are immature fish 1 inch or less in length which have absorbed their yolk-sacs; "fingerlings" are immature fish from 1 to 5 inches in length.

Spawners entering the streams where traps are in operation have been counted from 1944 to the present. The average numbers of spawners appearing in the various streams are arranged in table 2 by 2-week periods. Some streams support earlier runs than do others (Cope, 1957), Arnica Creek having the earliest spawning run and the warmest water in the late spring. Moreover, Arnica Creek post-spawners (adult fish that have spawned, survived, and are moving toward or have reentered the lake) are caught by fishermen in great numbers earlier than those from other streams. For each stream, a close relation exists between temperature characteristics (table 1), movements of spawners into the stream (table 2), and subsequent entry of spent fish into the lake sport fishery (appendix table 8).

Table 3 contains sex-ratio information from the spawning runs and the catch.

The sport fishery

The fishing season extends from June 15 (May 30, before 1953) to October 15 for the lake, and from July 1 to October 15 for the Yellowstone River from Fishing Bridge downstream to the falls. Fishing is not permitted in the tributary streams while spawning is in progress, but an overlap in time exists between the beginning of lake and river fishing and the end of the spawning runs (table 2). A creel census conducted by the Fish and Wildlife Service has divided the fishery

TABLE 2.—*Cutthroat trout entering spawning runs, expressed as biweekly percentages of average spawning runs*

Time of season	Arnica Creek 1950–53	Pelican Creek 1949–53	Chipmunk Creek 1949–53	Grouse Creek 1951–53	Clear Creek 1953	All Streams 1949–53
May 4–17	4. 5	0. 4	0. 0	0. 0	7. 1	1. 4
May 18–31	23. 3	13. 3	. 0	. 0	7. 9	11. 3
June 1–14	35. 9	20. 0	19. 5	22. 8	7. 9	23. 4
June 15–28	17. 0	30. 0	27. 4	19. 9	3. 0	22. 6
June 29–July 12	11. 7	29. 6	33. 4	36. 8	43. 5	27. 6
July 13–26	6. 2	6. 6	15. 9	16. 4	29. 9	12. 2
July 27–Aug. 9	1. 2	. 0	3. 8	4. 1	. 6	1. 5
Aug 10–23	. 1	. 0	. 0	. 0	. 0	. 0
Aug. 24–Sept. 6	. 1	. 0	. 0	. 0	. 0	. 0
Sept. 7–13	. 0	. 0	. 0	. 0	. 0	. 0
Average number of fish in spawning run	4, 170	15, 178	11, 249	8, 896	11, 323	10, 163
Average duration of run......days	78	46	57	53	72	60

Table 3.—*Ratios of females to males in spawning runs of cutthroat trout from Yellowstone Lake and in the catch*

IN SPAWNING RUNS

Stream	1945	1946	1947	1948	1949	1950	1951	1952	1953	Stream average
Arnica Creek	------	------	------	------	------	1.50	1.63	1.76	1.37	1.56
Pelican Creek	2.06	1.27	1.68	1.80	1.62	1.66	1.70	1.00	2.00	1.64
Clear Creek	1.39	1.95	1.19	1.08	1.52	1.36	1.12	1.20	1.89	1.36
Cub Creek	1.24	2.03	1.22	1.30	------	1.27	1.09	1.17	1.47	1.40
Chipmunk Creek	1.65	1.59	1.32	1.74	1.38	1.50	------	1.18	1.57	1.48
Grouse Creek	1.45	1.18	1.28	1.05	1.59	1.61	------	1.36	1.37	1.40
Year average	1.55	1.61	1.34	1.41	1.55	1.52	1.25	1.18	1.73	1.47

IN CATCH

Area of lake	1950	1951	1952	1953	Lake average
Fishing Bridge	0.72	1.20	1.56	1.60	1.29
West Thumb	1.21	1.83	1.58	3.21	1.95
Year average	.99	1.51	1.57	2.24	1.62

into several natural components with respect to type and area of fishing (Moore, Cope, and Beckwith, 1952; Cope, 1957a). This division includes angling from the West Thumb, Lake, and Fishing Bridge guideboats and rental rowboats, landings from the private cruisers and private rowboats, the shoreline and river fishing, and the famous Fishing Bridge fishery. The area covered by the shoreline fishery is largely controlled by the road which parallels the lakeshore from West Thumb to Cub Creek via Fishing Bridge. The section around Fishing Bridge, which spans the river at its outlet from the lake, is fished more heavily than any other location (Cope, 1957a). Tourist accommodations are largely centered in this area. Boat docks at Fishing Bridge, the Lake Hotel, and West Thumb offer rental rowboats and guideboats, and have facilities for the launching of private craft. The inaccessible southern half of the lake receives light fishing pressure from the cruisers and guideboats. West Thumb is moderately fished by shoreline and boat fishermen. The central part of Yellowstone Lake is not heavily fished. The locations of boat docks, access roads, and tourist accommodations are largely the cause of the unequal distribution of fishing.

From 1950 through 1957 the total catch estimate for Yellowstone Lake averaged 284,813 fish per year (table 4). Total harvest for the Yellowstone River averaged 25,964 per year from 1951 to 1957. The seasonal distribution of catches in units of the fishery appear in table 5, and the average contribution of each component of the creel census is summarized in table 6. The Fishing Bridge fishery accounted for less than 3 percent of the total average catch from the lake; private rowboats and the shoreline fishery were the most successful.

Table 4.—*Total catch estimates for Yellowstone Lake and River, 1950–57*

[In number of fish]

Year	Lake	River	Total
1950	200,015	(1)	(1)
1951	208,255	19,729	227,984
1952	245,277	20,603	265,880
1953	195,873	27,932	223,805
1954	215,933	35,912	251,845
1955	286,056	63,701	349,757
1956	290,221	21,907	312,128
1957	301,155	41,961	343,116

[1] No river census taken in 1950.

Table 5.—*Catches in various fisheries for certain years during tagging operations, expressed as percentages of average catch for each fishery*

Dates	West Thumb guideboats and rowboats 1950–53	Shoreline 1951–53	Fishing Bridge-Lake Dock guideboats and rowboats 1950–53	Fishing Bridge 1951–53	Yellowstone River 1951–53
June 1–14	4.4	10.6	3.0	------	------
June 15–28	11.0	25.7	9.6	------	------
June 29–July 12	17.7	24.5	22.7	38.9	29.3
July 13–26	20.1	21.4	21.1	34.8	29.0
July 27–Aug 9	19.8	8.3	17.8	14.4	14.0
Aug. 10–23	19.7	7.9	15.2	7.5	13.8
Aug. 24–Sept. 6	7.1	1.2	8.6	3.9	12.4
Sept. 7–13	.2	.4	2.0	.5	1.5
Average catch	25,058	63,919	48,629	6,158	22,755

Table 6.—*Average annual contribution of each component of the Yellowstone Lake fishery as determined by creel census from 1950 to 1955*

Fishery	Percentage of average lake catch
Rental row boats:	
Fishing Bridge-Lake Dock	11.4
West Thumb	9.2
Guideboats:	
Fishing Bridge-Lake Dock	11.5
West Thumb	2.6
Private boats:	
Rowboats	27.8
Cruisers	4.5
Shoreline	30.1
Fishing Bridge	2.9

METHODS

Nest and fry mortality

A study designed to estimate mortality of cutthroat trout eggs in the nest, and the average number of fry produced per nest, was made at Arnica Creek from 1951 through 1953. Mortality with respect to time through the end of the incubation period (terminal mortality) was measured. Observation of spawning activities of adults permitted the indentification of individual nests. A total of 58 redds was marked and carefully excavated at periods of time varying from 4 to 32 days following deposition. The eggs were collected in a wire screen at a point immediately downstream from the nest. The total number of dead and viable eggs in each nest was determined by count. Live eggs that had been damaged and killed as the result of digging could readily be identified when compared with normally deceased ova.

At the onset of hatching in Arnica Creek, fine-mesh screens were installed in the traps and on the racks to lead the downstream-migrating fry and fingerlings into the trap. These small fish were counted, measured, and released downstream. The trap was removed in late September, when a visual stream survey was made upstream to estimate the total number of fish of each size group remaining in the stream. The sum of the seasonal trap count and of those estimated to be remaining in the stream gave a measure of survival through the summer. The numerical difference between the calculated hatch of yolk-sac fry and the estimate of fish of the year alive in September has been termed "fry mortality", and covers the time from hatching (June–July) to the end of the summer (September-October). The egg potential was obtained by multiplying the number of females entering the stream by the average fecundity figure given by Welsh (1952)[5] and assumes complete deposition. To compensate for the action of predators, scavengers, and other sources of mortality that deprive the spawning run of its full egg potential, the highest nest mortality figure, 75 percent, rather than an average or minimum was used to compute the hatch of yolk-sac fry.

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[5] Welsh, J. P., A population study of Yellowstone blackspotted trout (*Salmo clarkii lewisi* (Girard). Ph. D. dissertation, Stanford University, 1952, 120 pp.

Tagging of adults

The fish traps in Pelican, Clear, Chipmunk, and Grouse Creeks have been operated for many years to collect eggs for fish-cultural purposes. Beginning in 1949 some spawners were tagged and then released upstream for natural reproduction. This program continued through 1953. A constant proportion for each stream (1 in 3, 1 in 7, etc.) was tagged, depending mainly upon the anticipated number in the run. Some tags were applied to adult fish at Pelican and Chipmunk Creeks in 1950 after the spawn had been taken by hatchery men. After tagging, the fish were placed immediately upstream of the trap, a standard technique employed by the Yellowstone hatchery employees.

A new method of estimating stream mortality was introduced in 1953. This procedure involved the tagging of postspawners, as well as prespawners. Because downstream traps were lacking at some wiers, seines and electric shockers were used in some streams to obtain the spent adults.

Arnica Creek (fig. 1) was established in 1950 as an experimental stream for the study of natural spawning. A combination upstream-downstream trap was constructed near the mouth to obtain total counts of upstream and downstream migrants. In the first year, spring floodwaters undercut the apron and rendered the upstream trap only partially effective. Later in the season the lake level rose and inundated the structure, allowing both downstream and upstream escapement. In 1951, all prespawners were caught in the upstream trap, but later the level of the lake rose again, flooded the trap, and allowed some fish to escape downstream.

The Arnica Creek trap was moved farther upstream in 1952 to eliminate further flooding. Again, spring floods and early summer rains interfered to allow a considerable portion of the downstream run to bypass the structure, but the upstream run was enumerated completely, as in 1951. No serious difficulties were experienced in 1953, and complete counts were made of upstream and downstream migrants.

Postspawners were tagged at Arnica Creek in 1953 as a new method of estimating spawning mortality. The total trap counts this year, which gave an absolute measure of spawning mortality, afforded a check on the estimate gained by the upstream-downstream tagging method.

Beaverdam Creek and a few smaller tributaries in the Southeast Arm were included in the tagging operations of 1952. Small portable traps were used at these locations, and only a limited number of prespawners were captured and tagged. Since few fisherman frequent this area, the return of tags from the fishery was negligible. These data are not considered in the present paper.

The fish were obtained at random from the traps and were anesthetized in chloretone (chlorobutanol) or urethane (ethyl carbamate). Although both rendered the trout immobile for tagging, urethane proved to be more desirable (Cope, 1953b). Best results were obtained with a 0.25 to 0.30 percent solution of urethane within a temperature range of 48° to 52° F. At lower temperatures the fish were affected so slowly that they injured themselves by excessive activity in the holding tank. This slow reaction also reduced the rate at which the fish could be handled. At higher temperatures the fish were affected so quickly that under continued exposure some were injured or even killed. Because of this effect and during the latter part of the spawning runs (table 2), tagging was limited to the early part of the morning, when water temperatures were within this favorable range.

Anesthetized fish were tagged, measured and weighed, and scale samples were taken. The tagged trout were then placed in live boxes until complete and normal recovery was indicated. Mortality immediately attributable to tagging and handling was negligible.

When postspawners were later recaptured in the downstream trap or caught during sampling of the spawning population in the stream adjacent to the trap, they were again weighed, their tags were checked and adjusted if necessary, and they were released. This second capture permitted the study of weight loss and length of time spent in the stream during spawning. A number of adults were tagged in Pelican and Chipmunk Creeks in 1950 after having been spawned by fish culturists. These fish were released upstream and some were recaptured through stream sampling. This procedure yielded comparisons between those fish reproducing naturally and those artifically spawned for spawning mortality, weight loss from spawning, and time spent in the stream.

Fish caught in a trap net fished in the lake about 1 mile south of Fishing Bridge were tagged in 1952 and 1953. The tagged trout were released in the area of capture.

From 1951 through 1953, adult cutthroat were tagged at the Cascades of the Yellowstone River, approximately 5 miles downstream from Fishing Bridge. The fish were obtained from a pool in the Cascades where they congregated while moving upstream. Capture was made by hand dip nets and electric shockers, and the fish were treated in the standard fashion before being released. Most of those taken proved to be prespawners.

The Petersen disk was the tag used exclusively throughout this study. Except for the tags used in 1949, an identification was carried on one of each pair of disks (inked, or stamped, or printed numerals). The color of the returned tag established the place of tagging, while the mark on the tag identified the individual fish.

Tags were recovered from several sources. A few were taken from dead fish in streams. Most of the tags were recovered on a semivoluntary basis from fishermen. Posters were placed at strategic points around Yellowstone Lake to inform the public of the presence of tagged fish and the need for their return by the fishermen. The cooperation of boat-dock employees and fishing guides of the Yellowstone Park Company and of National Park Service employees greatly facilitated the return of tags. Many tags were also recovered annually from nests at the white-pelican rookery at Molly Island in the Southeast Arm (fig. 1). The pelican is the principal predator on the cutthroat in Yellowstone Lake. Upon capturing a marked fish, the bird may return to the rookery and regurgitate the fish to feed the young pelicans, or may deposit the tag on the island in fecal material. Tags were recovered by searching in and around the nests.

Visitors to Yellowstone Park were interviewed in 1953 to determine the efficiency of the semivoluntary tag-return system. Road blocks were operated once a week throughout the fishing season at strategic locations near fishing areas. Automobiles were temporarily detained and all tourists were questioned about their fishing at Yellowstone Lake. A total of 26,031 people were questioned between June 21 and September 6.

Tag return areas

Migration of postspawners from the tributaries was measured from the distribution of tag returns from the sport fishery. The lake and river were divided into areas delimited by geographical or fishery characteristics, or both. These areas are indicated in figure 2, with pertinent geographical localities that are concerned with the tag return results.

The Fishing Bridge area is at the outlet of the lake. This area includes Fishing Bridge itself and extends about half a mile upstream and a mile downstream from this structure. The Yellowstone River area extends from the lowermost limit of the Fishing Bridge area downstream to the upper falls, a distance of about 14 miles. The section of Yellowstone Lake lying

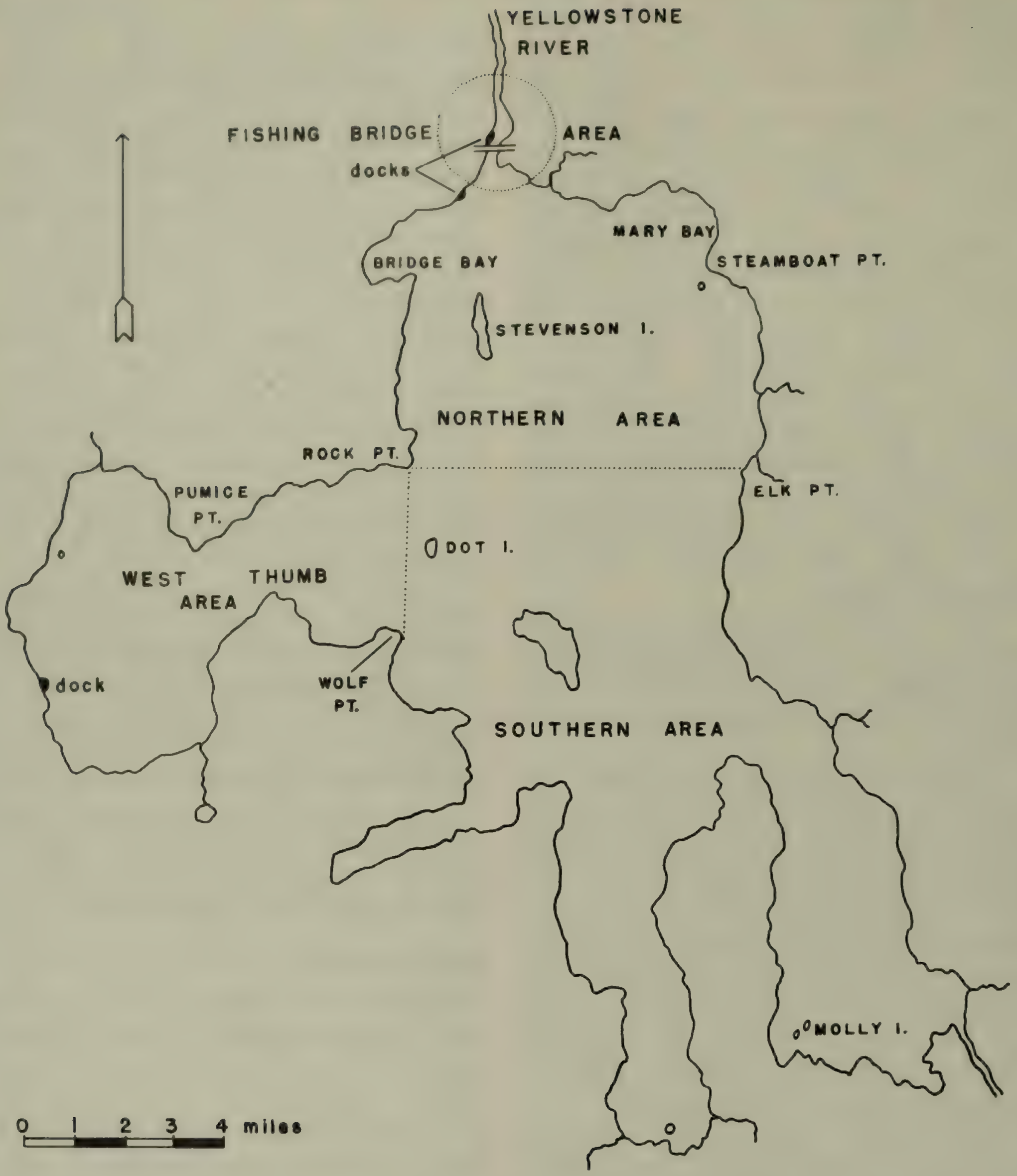

FIGURE 2.—Yellowstone Lake and River fishing areas and important geographical locations.

north of a line from Rock Point on the west shore to Elk Point on the east is termed the northern area. The part of the lake to the west of a north-south line connecting Rock Point and Wolf Point is designated the West Thumb area. The rest of the lake is included in the southern area, which incorporates the central part of the lake and Flat Mountain, South, and Southeast Arms.

Stream mortality of adults

Five methods of determining spawning mortality were considered in these studies:

1. Complete upstream and downstream trap counts (Arnica Creek, 1953).

2. Upstream and downstream tagging (Arnica Creek, 1953; Chipmunk Creek, 1952–53; Grouse Creek, 1952–53).

3. Sampling ratio, the downstream sampling of spawners that were tagged on

their way upstream (Arnica Creek, 1950–52; Pelican Creek, 1950; Grouse Creek, 1951).

4. Assumed fishing mortality (Pelican Creek, 1949; Chipmunk Creek, 1949).

5. Complete count of dead fish in the stream.

The complexity and variety of the sampling procedures necessitated the use of these several methods to produce comparable results. The following notations and definitions have been used in the analysis:

T	Tags applied to prespawners.
t	Tags applied to postspawners.
Nu	total adults migrating upstream (prespawners).
Nd	total adults migrating downstream (postspawners).
NT	total number of prespawners tagged NT/week=tag group.
Nt	total number of postspawners tagged Nt/week=time group.
$n(Td)$	sample of NT checked downstream through trap as postspawners $n(Td)$/week=time group.
$N(Td)$	total number of NT checked downstream through trap as postspawners (full availability to the fishery) per week=time group.
$(Tf);(tf)$	tags returned from the fishery.
sm	number of deaths in stream (stream mortality).
fmi'	instantaneous fishing mortality (a rate).
fma'	seasonal fishing mortality (a rate).
sm'	stream or spawning mortality (a rate).
lm'	natural mortality in lake (a rate).
s'	survival (a rate).
SR	sampling ratio (a factor).

The first method depends upon total upstream and downstream counts of spawning run fish. Tagging is not involved. Therefore, the equation is as follows:

$$sm = Nu - Nd$$

The second method is an estimate. Here, the downstream migration is composed of some fish that were tagged during upstream movement and others that were not. Untagged fish moving downstream are sampled and tagged (t) and released downstream to enter the fishery in mumbers approximately equal to the lakeward migration of tagged prespawners, $n(Td)$, in each sample. The method assumes subsequent equal availability in the lake of both $N(Td)$ and Nt, the known quantity. Further, we assume total availability of Nt to the fishery without significant delayed mortality due to tagging or spawning. Therefore:

$$\frac{N(Td)}{(Tf)} = \frac{Nt}{(tf)}$$

Solving for $N(Td)$

$$N(Td) = \frac{Nt(Tf)}{tf}$$

$$sm = NT - N(Td)$$

Evidence of a small mortality due to tagging, to be discussed later, tends to violate one of the assumptions upon which this method is based, and therefore limits its value to some extent.

The third method uses a factor termed the "sampling ratio," SR. As in the upstream-downstream tagging method of estimating stream mortality, random sampling of the downstream migrants containing T is involved. Subsequently, the tags returned from the fishery are used to compute the total downstream migration and the spawning mortality in the following manner:

$$SR = \frac{(Tf)\ (\text{not present in } n(Td))}{(Tf)\ (\text{present in } n(Td))}$$

then

$SR \times n(Td)$ (sampled) $= n(Td)$ (not sampled, escaped downstream); therefore,

$n(Td)$ (sampled) $+ n(Td)$ not sampled) $= N(Td)$

and

$$sm = NT - N(Td)$$

Before the use of upstream-downstream tagging, the sampling ratio was the principal method of estimating spawning mortality at all locations. Sampling took place below the upstream traps to ensure the taking of fish that had completed spawning. Visual inspection of fish obtained aided in this determination. Hence, the sampling ratio establishes a relationship between the number of fish that escaped sampling and those that were sampled. Obviously, the downstream collections must include a large enough number of tagged fish, T, so that the fishery tag returns will provide a reliable ratio of sampled and nonsampled tags. At Arnica Creek (1950–53) the downstream trap which operated inefficiently provided a large enough weekly sample to allow the SR to be applied to the $n(Td)$ for each time period. Following this procedure, if the SR computed from week to week varied significantly within the season, it was applied where appropriate. The $N(Td)$ for the season would then be equal to the summation of estimates for each time period. However, where the variation of the sampling ratio was small, as was the case in 1950 and 1951, the mean SR for the season was applied to each period's $n(Td)$. Postspawners at Arnica Creek apparently avoided the downstream trap at a constant rate in 1950 and 1951 due to early flooding by spring runoff and, later, by the rising of the lake level. In 1952, hampered only by the excessive spring runoff, the sampling rate varied. The small number of tags sampled at other streams restricted the application of the SR to only the total number of $n(Td)$ obtained for the entire season. If enough tags are obtained in the downstream sampling process, then both methods 2 and 3 may be simultaneously employed.

Because identification symbols were lacking on the tags used at Pelican and Chipmunk Creeks in 1949, a fourth method of estimating stream mortality was employed. The more reliable data from later years' tagging for any single tributary indicate that fishing mortality for postspawners varies less from year to year than does stream mortality. Assume, then, that the fishing mortality for each stream, as indicated by the return of tags from the fishery, is similar to an average estimate obtained from the more accurate data of other years. In order to approximate the number of tagged fish that survived spawning, the following relationship must be established where $(fm)a$ is an average estimate derived from data of other years:

$$\frac{(fm)a}{100}=\frac{(Tf)}{N(Td)}$$

solving for $N(Td)$

$$N(Td)=\frac{Tf\times 100}{(fm)a}$$

then

$$sm=NT-N(Td)$$

If all of the adults that died during stream residence could be observed by patrolling the spawning grounds, the tabulation would give total stream mortality. This method has been used in some Pacific salmon studies. However, at Yellowstone, because of the sizes of the tributaries, the rapid destruction of carcasses by scavengers, and the activities of predators, this approach did not give accurate results and was not used.

Mortality in the lake

The determination of mortality in Yellowstone Lake primarily concerned the postspawners returned to the lake from tributaries. Stream mortality estimates made these determinations possible. One method for mortality determination was used for trout from these five streams. Other methods were necessary for fish tagged at the Cascades of the Yellowstone River and at the trap-net site on Yellowstone Lake. At these locations samples were tagged without knowledge of the numerical relation to the population they represented. Furthermore, fish tagged at the

Cascades and at the trap net were immediately available to the fishery, if fishing was in progress at the time of tagging. These factors required modification of the method used for mortality estimates for trout from the tributary streams.

Much of the basis for the methods and definitions in this report is founded on the work of Graham (1938). In dealing with fishing mortality, he defined rate of fishing as the "catch in a moment of time expressed as a percentage of the stock at that moment." This involves the concept of instantaneity. The concept of fishing intensity was presented in terms of the summation effect of fishing season upon a stock of fish.

The methods developed in this report deviate in detail, but not in principle, from Graham's treatment. The influence of the fishery upon the stock of available tagged fish is here termed the intensity of fishing. This may be considered from two viewpoints, the instantaneous (rate of fishing, *(fm)i*) and seasonal (fishing mortality, *(fm)a*) rates.

The rate of fishing is measured in relation to the accumulation of tags available to the fishery (cumulative availability) during a weekly period (time period). The fishery tag returns during that period are expressed as a percentage of the cumulative number available. While Graham dealt with the average number of marked fish available during a unit of time, we used, instead, the total number of available fish accumulated to the end of the unit of time. This was considered to be most desirable, since tagging and escapement to the fishery occurred before and during the most intense fishing period. Also, instead of treating instantaneous mortality in terms of number of tags liberated per instant of time and simultaneous relation to returns, as has been suggested by Ricker (1948), the analysis was based on the accumulation of tags throughout the summer season, notwithstanding limitations imposed by concurrent, natural mortality. This method is considered to provide a more accurate measure of the effects of fishing where numbers of returns were small. The rate of fishing, therefore, may best be defined as the number of tagged fish captured by fishermen within an instant of time (weekly period) divided by the accumulated available tag population tabulated to the end of that instant of time.

The rate of fishing may also be expressed as an instantaneous fishing mortality per 100 fish available within a period of a week. This value can be assumed to approach the rate of exploitation affecting the total available fishable population within the scope of tag dispersion. The concurrent effect of natural mortality (the rate of which has not been determined) cannot be isolated in this treatment; therefore, the resulting errors cannot be eliminated.

Rate of fishing is also presented in a slightly different light, and is termed "period fishing returns" or "rate of tag return." This method, involving a slightly different concept of time, is intended to render the less complete data comparable with information from all tagging locations and with the creel census catch statistics. The period fishing returns relate the number of tags identified with a particular stream or area returned within a time period to the season's total for that population. The statistic is expressed as a percentage of the total for the season.

Seasonal fishing mortality *(fm)a* is treated by two methods. The first method deals with the survivors of an upstream group tagged during a time period and with the subsequent returns from the fishery. This requires precise data involving tag group stream mortality rates for each time period and has been used only at Arnica Creek. The measure is called "tag group fishing mortality." It represents the relation between the survivors of an upstream tag group processed within a time period and the subsequent returns for a fishing season. Stream mortality and biology are intervening variables

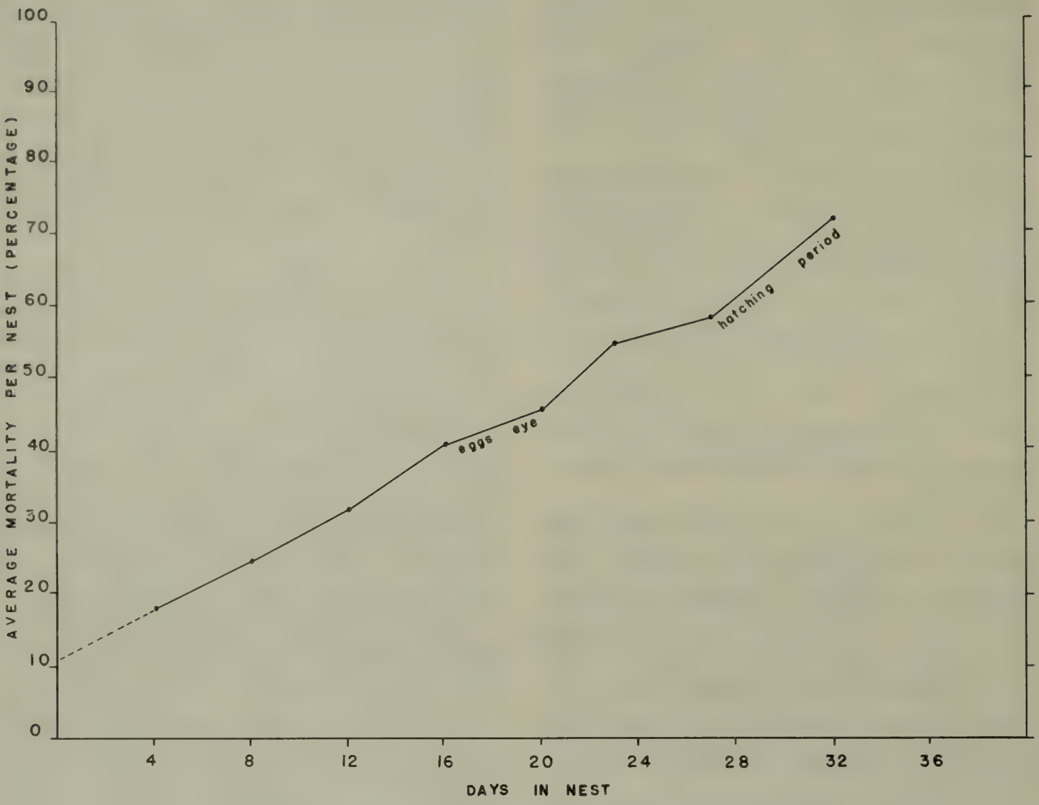

Figure 3.—Mortality to eggs from deposition to hatching.

that determine group availability and catch.

The other method is termed "time group fishing mortality." The approach is concerned with tags actually checked downstream to the fishery during any given time period, a time group, and the subsequent fishery returns during the season. Stream mortality and biology have been eliminated as variables because this tag unit originates during each week as a portion of the downstream migration. It was either totally enumerated (Arnica, 1953) or adequately sampled (Arnica, 1950–52). At other stream locations where downstream sampling was inadequate, this method was not applicable. At the trap net and Cascades, the tag and time group are synonymous, since the fish were immediately available to the fishery at the trap net and were practically so at the Cascades. When considered on a seasonal summary basis, both methods of evaluating fishing mortality of tag groups or time groups are identical; the principal difference between these two methods lies in the identification of the tag units.

RESULTS

Egg, fry, and fingerling mortalities

The results of the egg mortality measurements at Arnica Creek (1950–53) are summarized in table 7. A total of 58 nests was examined, almost equally divided between 1952 and 1953. Only six nests were included in 1951. The sampling of nests with respect to time was accomplished in proportion to the intensity of spawning, as much as possible. Since there were no discernible differences between the results of 1952 and those of 1953, all data

TABLE 7.—*Summary of egg mortality data, Arnica Creek, 1951–53*

Days in nest	Development events	Average number of eggs and fry	Number of nests sampled	Number of eggs		Percent dead	
				Alive	Dead	Mean	Range
4		423	7	349	74	17. 5	1. 3–30. 8
8		283	8	215	58	24. 0	5. 0–45. 7
12		349	8	238	111	31. 5	6. 6–40. 1
16	Eggs become eyed	354	8	211	143	40. 4	6. 9–100
20		422	10	231	191	45. 3	9. 9–62. 2
22-24		410	10	152	225	54. 9	11. 0–100
26-28	Hatching starts	319	7	135	184	57. 7	4. 5–100
32		229	3	57	172	75. 1	63. 0–100
36-40	Hatching completed	56	3	3	161		
Season		369	58				

TABLE 8.—*Summary of fry mortality data, Arnica Creek, by years, 1950–53*

	1950	1951	1952	1953	Average
Egg potential	2, 578, 203	3, 263, 436	2, 485, 215	2, 904, 768	2, 807, 905
Yolk-sac fry (25 percent of egg potential)	644, 551	815, 859	621, 304	726, 192	701, 976
Number of fry and fingerlings	27, 712	13, 488	597	4, 384	11, 545
Percentage of hatch	4. 29	1. 65	. 09	. 60	1. 64
Percentage of egg potential	1. 07	. 40	. 002	. 15	. 40
Mortality to September (percent of egg potential)	98. 93	99. 60	99. 97	99. 85	99. 60

were combined. Figure 3 depicts the mortality rate of eggs in the nest and is based on the information contained in table 7. The terminal nest mortality ranged from a minimum of 60 to a maximum of 75 percent. To compensate for the action of predators and other mortality sources that deprive the spawning run of its full egg potential, the apparent maximum nest mortality, rather than an average nest mortality, was applied. The results depict the number of yolk-sac fry estimated to have hatched.

Table 8 compares each year's production of yolk-sac fry in Arnica Creek with the estimated number of immature fish surviving in September. The difference between these two quantities is termed fry mortality.

The average nest mortality at the end of the first 4 days was 17.5 percent. Thereafter, the average mortality increased at the rate of about 6 to 7 percent for every 4 days through the 32nd day of incubation. The period of incubation here includes embryological development to hatching, but not into the yolk-sac stage. Based on the presence of yolk-sac fry in the gravel, hatching apparently began during the period from the 28th through the 32d day of incubation. As water temperature increased through the spawning period, time required for incubation decreased.

The results of these studies indicate an average time-mortality relation which approximates a straight-line function. It might be expected that a significant decrease in the mortality rate would be detected around the end of the third week in the nest. At that time the delicate stage of embryonic development is completed and the eggs become eyed. Incubation of cutthroat trout eggs at the Yellowstone hatchery has demonstrated that such a decrease is the rule rather than the exception. A decrease in mortality rate did not occur in the Arnica Creek studies. However, eggs in the hatchery are mechanically jarred (bumped) at the onset of the eyed stage. This operation is intended to induce an immediate mortality of the eggs that otherwise might die during the remainder of the incubation period. Hatchery-water temperatures are relatively constant without daily or seasonal fluctuation, and provide a stable environment throughout the developmental period. These artificial conditions are not duplicated in nature where the eggs are usually not mechanically disturbed in the nest, and diurnal and seasonal temperature variations occur. These conditions likely contribute to the observed uniform mortality rate of eggs in nature.

Extrapolation of the mortality curve (fig. 3) to the zero time point, which assumes a constant mortality rate from egg deposition, suggests an initial loss of approximately 10 percent. Other salmonid nest studies (Briggs, 1953) have shown fertilization to have efficiencies of 94–98 percent. It is suggested that if an initial loss of 10 percent is the case in the present studies, it may be attributable to both mechanical injury of eggs during spawning and lack of complete egg fertilization. Other sources of egg mortality that may operate to various degrees throughout incubation include high water temperature, disease, redd exposure due to lowering of water level, exposure due to superimposition, invertebrate predation, and silting of the redds.

The total number of eggs (dead and alive) uncovered from the 58 nests involved in this study amounted to 21,398. Disregarding deterioration of dead eggs (Briggs, 1953), the average number of eggs per redd was 369, with a range of from 229 to 423. The average fecundity of the Arnica Creek spawners in 1950 and 1951 was 1,107 eggs per female, with the smaller females (290 mm. or less in length) harboring 898 eggs and the largest size group having 1,296 eggs per female (Welsh, 1952). Using these independent averages, the results indicate that each female must prepare three nests in order that all of her eggs be deposited.

After hatching, the ensuing mortality measurement extends from the yolk-sac stage through the fry and fingerling stages in the fall of the year. From 1950 through 1953, an estimated average of 701,976 yolk-sac fry were produced yearly in Arnica Creek (table 8). Subsequent survival of fry and fingerlings to September varied from less than 1 percent (1952 and 1953) to 4.3 percent (1950). Considerable variation from year to year is evident and should be expected in this production. Of the 4-year total of 46,181 immature fish enumerated in September, 60 percent were accounted for in 1950 alone, and almost 90 percent in 1950 and 1951 combined. As a rule, 1.6 percent of the yolk fry production, or 0.41 percent of the egg potential, survived through the critical summer period. This amounts to an average fry sac mortality estimate of 98.4 percent (95.7 to 99.9 percent). There was no apparent relation between numbers of females involved in natural reproduction at Arnica Creek from 1950 to 1953 and the resulting production of fry. Similar negative relations have been detected in several salmon populations (Barnaby, 1944; Foerster, 1945).

Other studies of salmonid survival have also noted considerable variation between individual nests both within a single sampling area and from year to year. Survival for the Lahonton cutthroat trout (*Salmo clarki henshawi*) from the egg to the fry stage (3 months after emergence from the gravel) was shown to be 16 to 17 percent in 1 year. In the 2 years that followed, survival varied from 0 to 6 percent (Smith, 1947). Small numbers of nests and fry were used in these tests. In the same study, the survival of brook trout (*Salvelinus fontinalis*), measured from egg deposition in November to the following August, ranged from 3.6 to 42.4 percent and averaged 16.4 percent for 6 sampling units. Briggs (1953), with methods similar to those used at Arnica Creek, found an average terminal nest mortality of 35.1 percent for steelhead trout eggs, with great variations between samples. Comparable mortality figures for king salmon (*Oncorhynchus tschawytscha*) and silver salmon (*O. kisutch*) of 14.0 and 25.7 percent, respectively, were also listed. A tabulation of nest mortalities from various studies cited in the reports of Briggs, (1953)

TABLE 9.—*Summary of salmonid nest mortality data from Briggs (1953), Shapovalov and Taft (1954), and the present study*

Species	Authority	Number of redds sampled	Terminal mortality
			Percent
Data from Briggs:			
Red salmon	Chamberlain (1907)	58	("two-thirds in good condition").
Brook trout	White (1930)	1	21.0.
Do	Hazzard (1932)	[1] 21	19.2.
Red salmon	Foerster (1938)		30.0.
Coastal cutthroat	Cramer (1940)	[1] 4	95.2.
Atlantic salmon	White (1942)	1	22.0.
Chum salmon	Hunter (1948)	[2] 10	86.3.
Pink salmon	Hunter (1948)		96.8.
Brown and rainbow trout and king salmon	Hobbs (1948)	711	8.6.
Data from Shapovalov and Taft:			
Silver salmon	Harrison (1923)		25.0.
Do	Carl (1940)		46.3.
Do	Shapovalov and Berrian (1940)		89.8 (heavy silting).
Do	Shaw and Maga (1943)		83.8 (some silting).
			98.8 (artificially introduced mining silt).
Do	Foerster (1944)		88.6.
			88.2.
			69.6.
			74.0.
			74.4.
			60.0.
			59.9.
			83.7.
Steelhead trout	Shapovalov (1937)		71.2. (silting).
			20.1 (no silting).
Arnica creek data:			
Cutthroat trout	Present paper	58	60–75.

[1] Prehatching.

[2] Distributed between chum and pink salmon.

and Shapovalov and Taft (1954) provides data comparable with those from Arnica Creek (table 9).

Terminal nest mortality at Arnica Creek, 60 to 75 percent, appears to be similar to the average of those indicated from most other salmonid studies. However, two factors should be considered and weighed in order to make nest and fry mortalities significant, comparable, and objective. These items are the fecundity of the species and mortalities through the various developmental stages, particularly during the most critical segments of the life history. For instance, at Yellowstone Lake, in order to maintain numerical equilibrium in the spawning runs over a period of time, each mature female is responsible for the ultimate production of 1.67 adults, because of the unequal sex ratio in the spawning runs. To meet this requirement, a 0.15 percent survival from the 1,107 eggs per average female is necessary. Survival as high as 0.41 percent from egg potential to the stages in September has been measured at Arnica Creek. That is, 4.54 young trout per female had been produced to this point. Only 1.67 of these are required to reach maturity if the run is to remain in numerical stability. Thus, over a period of 3 years, which covers normal elapsed time to sexual maturity, 3.33 (63.20 percent) of the 5 immature fish alive in September will succumb. The survival rate of 36.80 percent suggests very favorable environment for the preadult period in the lake.

Many factors may influence the success of natural reproduction of cutthroat trout and survival during early developmental stages at Yellowstone Lake. They are:

1. Size of the spawning run (egg potential).

2. Capacity of the spawning gravels to accommodate spawners without occurrence of superimposition.

3. Predator population levels.

4. Relation between the advent of unfavorable environmental conditions in the stream and the onset, peak, and completion of spawning. The survival of adults, eggs, and fry is sometimes determined to a great extent by this relation (for example, lethal stream temperatures,

redd exposure due to lowering of stream level, silting and scouring of spawning gravels).

5. Log jams and other barriers of a temporary or permanent nature that render upstream spawning areas inaccessible and may result in superimposition in overcrowded downstream reaches.

6. Ability of the nursery areas to produce a sufficient supply of usable food for the newly hatched fry. The availability of food for young fish is especially critical at and shortly after the time when the yolk becomes absorbed.

7. Intrusion of temporary barriers (low water, hot springs) that might prevent free and uninhibited movement of fry from the spawning gravels to suitable nursery areas.

Stream biology and spawning mortality

The mortality to spawners during their sojourn in the spawning stream has been the subject of considerable attention during these studies. It is at this time of the life history that cutthroat trout are assembled in close quarters on the upstream and downstream migration, and the biologist is afforded the best opportunity for obtaining information on various aspects of its biology. In the section on Methods (p. 7), explanation was made of various procedures in measuring mortality to adults in streams. The results of the applications of these methods and of modifications used for mortality due to tagging are treated in the sections to follow.

Stream mortality of tagged vs. untagged trout.—The stream mortality estimates at Yellowstone have been examined in the light of possible differential mortality due to tagging. The anaesthesia, handling, and tagging were suspected of causing weakness in the tagged fish, spoiling the assumption that tagged fish acted exactly as did untagged fish.

The facilities at Arnica Creek were used to compare the upstream mortalities of groups of tagged and untagged trout from 1950 to 1953. Table 10 summarizes mortality for each group, and shows that tagged fish sustained mortalities of from 35 to 50 percent while in the stream. Untagged trout incurred stream mortalities ranging from 25 to 44 percent. Tagging imparted a maximum of 12 percent additional mortality on the spawners (1951), and a minimum differential of 4 percent (1950). The tagging mortality in Arnica Creek may also have been due to the impediment of wearing the tag during the migration, nest digging, and spawning. Individual differences in tagging and handling techniques among personnel could have contributed to year-to-year variations in mortalities between tagged and untagged fish.

TABLE 10.—*Differential stream mortalities at Arnica Creek, by years, 1950–53*

Cutthroat trout	1950	1951	1952	1953
Tagged vs. untagged:				
Tagged fish mortality (percentage)	38.0	41.2	35.3	50.3
Untagged fish mortality (percentage)	33.6	29.1	25.6	44.2
Differential mortality (percentage)	4.4	12.1	9.7	6.1
Chi square value	[1] 6.198	[2] 62.089	[2] 32.162	[2] 11.644
Male vs. female:				
Male sample mortality (percentage)	18.1	27.0	22.3	60.1
Female sample mortality (percentage)	18.9	26.0	25.3	44.6
Differential mortality (percentage)	.8	1.0	3.0	15.5
Chi square value	[3] .0304	[3] .6370	[3] 1.1117	[2] 11.397

[1] Significant (0.5 level).
[2] Highly significant (0.01 level or better).
[3] Not significant.

Mortalities in streams.—The test stream, Arnica Creek, was used for stream mortality measurements from 1950 to 1953, and afforded a convenient means for comparisons between methods in years when more than one method was used. Table 11 shows, for instance, that in 1953 two methods were used; these will be compared later. The table also shows the methods used for mortality estimates for other spawning populations and for trout in Yellowstone Lake.

Table 12 summarizes the corrected and uncorrected counts of trout through the Arnica Creek trap upstream and downstream, for the years 1950–53. Table 13 is a summary of all stream mortality estimates made from 1949–53.

The most reliable mortality estimates made were those on Arnica Creek. In one year, 1953, there was no escapement past the trap in either direction, so perfect counts were obtained. In years when some trout evaded the trap, corrections were made (table 12) from the results of a marking program.

TABLE 11.—*Summary of methods used in evaluating stream and fishing mortality*

Area and year	Stream mortality				Fishing intensity			
	Sampling ratio	Upstream-downstream tagging	Complete count	Assumed fishing mortality	Period returns	Tag group	Time group	Rate of fishing
Arnica Creek:								
1950	X	----	----	----	X	X	----	X
1951	X	----	----	----	X	X	----	X
1952	X	----	----	----	X	X	----	X
1953	----	X	X	----	X	X	X	X
Pelican Creek:								
1949	----	----	----	X	X	----	----	----
1950	X	----	----	----	X	----	----	----
1951	X	----	----	----	X	----	----	----
1952	X	----	----	----	X	----	----	X
1953	X	----	----	----	X	----	----	X
Chipmunk Creek:								
1949	----	----	----	X	X	----	----	----
1950	X	----	----	----	X	----	----	----
1952	----	X	----	----	X	----	----	X
1953	----	X	----	----	X	----	----	X
Grouse Creek:								
1951	X	----	----	----	X	----	----	----
1952	----	----	----	----	X	X	X	X
1953	----	----	----	----	X	X	X	X
Cascades:								
1951	----	----	----	----	X	X	X	X
1952	----	----	----	----	X	X	X	X
1953	----	----	----	----	X	X	X	X
Trap net:								
1952	----	----	----	----	X	X	X	X
1953	----	----	----	----	X	X	X	X

TABLE 12.—*Corrected and uncorrected trap counts at Arnica Creek, by years, 1950–53*

Tagged fish	1950	1951	1952	1953	Total
Upstream:					
Number tagged	965	1, 252	958	1, 019	4, 194
Number untagged	[1] 2,916	3, 503	2, 565	3, 505	12, 489
Total count	3, 881	4, 755	3, 523	4, 524	16, 683
Sampling ratio (SR)	3.15:1	1.82:1	[2] 0.3–5. 0:1	------	------
Percentage tagged	24. 9	26. 3	27. 2	22. 5	25. 1
Downstream sampled:					
Tagged fish	144	261	232	506	1, 143
Untagged fish	559	1, 153	869	1, 956	4, 537
Total	703	1, 414	1, 101	2, 462	5, 680
Downstream estimated:					
Tagged fish	598	736	620	506	2, 460
Untagged fish	1, 936	2, 484	1, 908	1, 956	8, 284
Total	2, 534	3, 220	2, 528	2, 462	10, 744
Stream mortality:					
Tagged fish	367	516	338	513	1, 734
Untagged fish	980	1, 019	657	1, 549	4, 205
Total	1, 347	1, 535	995	2, 062	5, 939
Total:					
Upstream run	3, 881	4, 755	3, 523	4, 524	16, 683
Downstream run	2, 534	3, 220	2, 528	2, 462	10, 744
Upstream mortality	1, 347	1, 535	995	2, 062	5, 939

[1] Estimated from marking of 764 in upstream trap count.

[2] Variable between these limits.

TABLE 13.—*Summary of stream-mortality estimates of spawners* (sm), *by years, 1949–53*

[Expressed as percentage values]

Stream	In tag population of—					Mean
	1949	1950	1951	1952	1953	
Arnica Creek	------	38. 0	41. 2	35. 3	50. 3	41. 2
Pelican Creek	65. 2	60. 8	29. 2	21. 0	30. 5	41. 3
Chipmunk Creek	23. 0	65. 7	------	94. 9	48. 0	57. 9
Grouse Creek	------	------	44. 2	58. 9	70. 1	57. 7
Clear Creek	------	------	------	------	41. 2	41. 2
Mean	44. 1	54. 8	38. 2	52. 5	48. 0	48. 1

Stream mortalities reported for other streams (table 12) were computed by the other methods indicated in table 11 and described in the section on methods. These methods are all dependent on the recovery of tags from postspawners in the lake and river fishery. Table 14 is a general summary of tags representative of postspawners from various streams and returned from the fishery. The "assumed fishing mortality" method used on Pelican and Chipmunk Creeks for 1949, giving mortality values of 65.2 and 23.0 percent, respectively, while not yielding statistically reliable results, nevertheless affords the most reasonable approximation possible under the circumstances.

The upstream-downstream tagging method gave reliable estimates in some instances. At Arnica Creek, this method was tested against a complete upstream and downstream trap count in 1953. The stream mortality estimate made by upstream-downstream tagging was 52.2 percent, compared with 50.3 percent for the actual count. In this case, 29 out of 353 downstream tags were recovered in the fishery in the current season. On other streams where the method was used in

TABLE 14.—*Summary of recoveries from the fishery of fish tagged in streams*

Stream and year of tagging	Percent of total returns in—										Total number of returns	
	Yellowstone River area		Fishing Bridge area		Northern area		Southern area		West Thumb area			
	First year	Second year	First year	Second year	First year	Second year	First year	Second year	First year	Second year	First year	Second year
Arnica Creek:												
1950	4.5	--------	9.1	7.7	22.7	15.4	4.5	7.7	59.1	69.2	44	13
1951	2.5	--------	8.6	--------	12.3	50.0	--------	16.7	76.5	33.3	81	12
1952	--------	--------	5.7	--------	22.9	50.0	2.9	--------	68.6	50.0	35	2
1953	2.9	--------	--------	6.7	14.5	20.0	7.2	6.7	75.4	66.7	69	15
All years	2.7	--------	5.7	4.8	16.6	28.6	3.5	9.5	71.6	57.1	229	42
Pelican Creek:												
1949	--------	--------	30.8	21.4	46.2	71.4	7.7	--------	15.4	7.1	26	14
1950	5.6	--------	37.0	11.1	57.4	55.6	--------	11.1	--------	22.2	54	9
1951	2.9	--------	11.4	29.2	74.3	62.5	4.3	--------	7.1	8.3	70	24
1952	3.5	--------	27.1	--------	64.7	92.3	3.5	--------	1.2	7.7	85	13
1953	1.0	4.3	19.2	8.7	66.3	52.2	6.7	13.0	6.7	21.7	104	23
All years	2.7	1.2	23.3	15.7	64.6	65.1	4.4	4.8	5.0	13.3	339	83
Clear Creek 1953	--------	--------	11.6	--------	62.8	54.5	11.6	18.2	14.0	27.3	43	11
All years	--------	--------	11.6	--------	62.8	54.5	11.6	18.2	14.0	27.3	43	11
Chipmunk and Grouse Creeks:												
1949	--------	--------	--------	--------	9.1	23.5	27.3	17.6	63.6	58.8	11	34
1950	5.9	--------	--------	--------	23.5	33.3	41.2	41.7	29.4	25.0	17	12
1951	--------	--------	--------	--------	32.1	--------	46.4	73.3	21.4	26.7	28	15
1952	--------	20.0	--------	--------	50.0	20.0	33.3	40.0	16.7	20.0	12	5
1953	--------	--------	--------	--------	19.0	20.0	61.9	60.0	19.0	20.0	21	5
All years	1.1	1.4	--------	--------	27.0	19.7	44.9	38.0	27.0	40.8	89	71

1953, less reliable estimates were obtained because of a paucity of returns from the fishery. From Chipmunk Creek, only 5 out of 112 tags were recovered; from Grouse Creek, 7 out of 172; from Pelican Creek, 2 out of 77; from Clear Creek, 19 out of 595. The reliability of stream mortality estimates by this method depends on a high return of tagged fish from the fishery, and on tagging and release of the downstream migrants at the same time that those not being tagged are passing downstream.

The other technique used at Yellowstone for estimating stream mortality, the sampling ratio method, was employed on four streams. The reliability of this method is also dependent on an adequate harvest of tagged fish. At Pelican Creek in 1953, there were 114 returns from 1,246 fish tagged, giving a more reliable estimate; the figure for Chipmunk Creek in 1951 may be questionable, since only 19 tags from 1,491 tagged fish were returned.

Spawning mortality at stream locations other than Arnica Creek has suggested significantly high death rates. These data lack the reliability of the information derived from Arnica Creek. Because differential mortality estimates pertaining to tagged and untagged spawners are not available from other areas, the spawning mortality values presented in later summaries are derived from tagging data only and are therefore comparable only for the tagged populations. Hence the figures probably represent maximum estimates and could be from 4 to 12 percent high.

Pelican Creek, a moderately warm stream with a large drainage area, supports a relatively early spawning migration. Stream mortality averaged 41.3 percent for the period 1949–53, and great fluctuation about this mean figure occurred. It is noteworthy that the two years of lowest survival of spawners at Pelican Creek were 1949 and 1950, when the two largest spawning runs were tabulated.

Both Grouse and Chipmunk Creeks also averaged more than 50 percent spawner mortality. It is remarkable that the averages computed during the study period for both streams, notwithstanding independent year-to-year variations, were exactly the same, 56.8 percent. However, the most obvious sources of spawning mortality operating in Arnica and Pelican Creeks, high water temperatures and pollution, are not duplicated in these two tributaries to the South Arm. Predation by white pelicans seems to act as the principal mortality source in this area. The pelican-trout relation will be discussed later.

The tagging operations at the trap net and Cascades depended on spawning migrations passing these two locations. Shortly after tagging, the fish were characteristically taken in the area around Fishing Bridge. Less than 10 percent of the tags applied at the trap net later appeared at the trap on Pelican Creek, which is the spawning tributary nearest the trap net location. Large quantities of excellent spawning gravel are present for a distance of half a mile upstream and a mile downstream from Fishing Bridge. Trout build nests in this area during June and early July, and large numbers of fry are seen there later in the season. Sexually mature fish, many of which are prespawners, dominate the Fishing Bridge fishery. This direct and indirect evidence is the basis for concluding that the fish tagged at the Cascades were moving up the Yellowstone River to spawning gravels around Fishing Bridge. The trap net also sampled mature "lake" fish, most of which were leaving the northern section of the lake for the same spawning area near Fishing Bridge.

The migration of trap-net fish to the Fishing Bridge spawning gravels began during the latter half of June and reached a peak during the last week of the month. Adult "river" trout appeared to be moving up the Yellowstone River earlier in June, at least before the migration of "lake" fish past the trap net location was detected. Maximum movement of river fish took place early in July, indicating a more extended spawning run. Both the opening of the lake fishery (May 30 or June 15) and the onset of fishing in the river and on Fishing Bridge (July 1) exerted a profound influence over the reproductive activities in this particular area.

Adult fish tagged at traps in smaller streams were not available to the fishermen until spawning was completed and the survivors had returned to the lake. On the other hand, fish from the trap net and Cascades tagging operations were exposed to the fishery before, during, and after spawning activities. For this reason the estimate of lake mortality (*lm*) for the last two groups of tagged fish also includes some cumulative effects of spawning mortality.

The Fishing Bridge area fishery has some characteristics that are similar to those detected at Grouse and Chipmunk Creeks. In the South Arm, the white pelicans prey heavily on adult fish in the stream during spawning. Similarly, angling in the Fishing Bridge area depends on adult cutthroat as they approach the spawning gravels, are in the process of nest building, or as postspawners. Observations strongly suggest that in both locations, a significant portion of the harvest by predators and fishermen consists of prespawners.

Stream mortalities and sex.—With one exception, male and female spawners sustained similar spawning mortalities. The reason for a mortality difference between sexes in Arnica Creek in 1953 may relate to temperature. That year, spawning mortality was significantly higher than in previous years (table 10). Mean maximum period temperatures in the stream reached the critical stage, around 65° F., early and were sustained at this level over a longer period of time than in any of the other years at Arnica Creek (fig. 4). This year, the male spawners spent two days more than the average time in the stream (table 15), and an unusually large number of bears frequented the area. These three conditions may have increased the probability of the male population experiencing a significantly higher stream mortality than the female. Presumably these con-

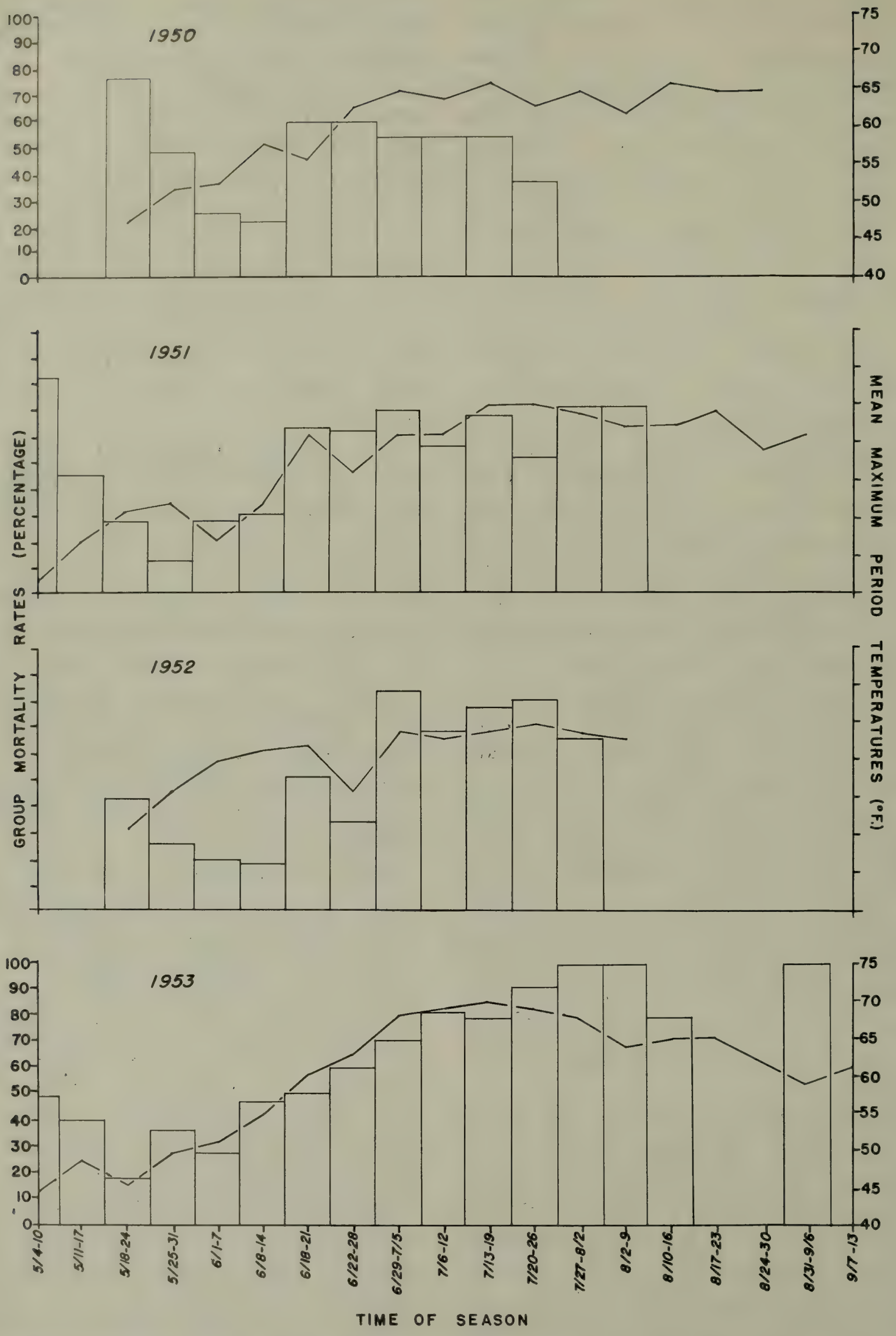

FIGURE 4.—Arnica Creek tag group mortality rates (histograms) and mean maximum period temperatures plotted against time period, 1950 to 1953.

TABLE 15.—*Weight loss and spawning time for tagged trout in Yellowstone streams, 1950 to 1953*

Year, stream, and number of tagged trout	Average loss in weight (ounces)	Average time upstream (days)	Quality of sampling
1950:			
Arnica Creek:			
49 males	3.2	20.3	Good.
73 females	3.9	11.5	
Pelican Creek, artifically spawned:			
5 males	3.2	8.2	Fair.
47 females	2.4	8.9	
Chipmunk Creek, artificially spawned:			
43 males	2.2	9.9	Good.
74 females	2.7	6.3	
Pelican Creek, natural reproduction:			
38 males	3.9	23.3	Fair.
24 females	3.8	21.0	
Chipmunk Creek, natural reproduction:			
48 males	----------	20.2	Good.
59 females	----------	13.1	
1951:			
Arnica Creek:			
92 males	3.2	17.4	Good.
144 females	3.8	10.5	
Pelican Creek:			
1 male	2.5	23.0	Poor.
10 females	2.8	11.4	
Grouse Creek:			
1 male	4.0	31.0	Poor.
1 female	1.0	10.0	
1952:			
Arnica Creek:			
50 males	2.5	12.3	Good.
146 females	2.5	6.0	
1953:			
Arnica Creek:			
159 males	2.4	19.2	Good.
394 females	3.0	12.0	
Pelican Creek:			
4 males	2.4	18.5	Poor.
5 females	2.6	20.2	
Grouse Creek:			
5 males	2.2	18.4	Fair
11 females	2.8	10.2	
Chipmunk Creek:			
2 males	2.5	35.0	Poor.
0 females	----------	----------	
Clear Creek:			
6 males	3.2	17.1	Poor.
22 females	4.4	18.7	

ditions were not critically accentuated in other years to a degree where the effect was differentially applied.

Female spawners in Arnica Creek lost an average of 2.5 ounces in 1952 to 3.9 ounces in 1950 during stream residence, while the male losses ranged from 2.4 ounces in 1953 to 3.2 ounces in 1950 and 1951. The 4-year average showed a 3.3-ounce weight loss for females and a 2.8-ounce loss for males, a difference of almost half an ounce.

Sex ratios.—Sex ratios in the spawning migrations in tributaries to Yellowstone Lake are somewhat unusual in their predominance of females. Table 3 is based on sex determination of 193,305 males and 284,893 females in 6 tributaries at Yellowstone from 1945 to 1953, and on several thousand mature fish caught at random from Yellowstone Lake. The data clearly show that females consistently outnumber males in the migrations and in the catch. The male-female ratio for combined data from the spawning runs is 1:1.47, and for the fishery, 1:1.62.

The phenomenon of a female-dominated sex ratio in the spawning runs at Yellowstone would appear to be important in regard to reproduction potential. There is, however, no evidence that the uneven sex ratio is detrimental to the maintenance of the population of the cutthroat trout.

The reason for the development of the unequal sex ratios in the spawning runs at Yellowstone Lake remains to be explained. It is assumed that the sex ratio of those fry emerging from the gravel is 1:1. Then, at some later time preceding their maturity, either a differential mortality occurs, or the sexes do not enter the streams for spawning in equal numbers. It has been suggested by other workers (Barnaby, 1944) that at the onset of the spawning migration, when the prespawners are approaching the tributary streams and are vulnerable to the fishery, the male may be more susceptible to the fishery. It is well known that male salmonoids are particularly aggressive during the time of reproduction. The thought has been advanced that they may be especially easy to catch at this time. This possibility was explored in an analysis of the sex ratios of the fish during the early portion of the fishery. While a significant predominance of males was noted early in the season, the females outnumbered the males for each season, 1950 to 1953. The total catch involved could not possibly account for the unequal ratio detected later at the traps. If this early fishery was mostly the cause of the differential harvest, then the heavier fishing pressure directed against the northern and West Thumb areas should result in much stronger spawning-run ratios in favor of the females in these areas, compared with those of the southern half of the lake.

Actually, a suggestion of higher female-to-male ratios in Pelican and Arnica Creeks over Chipmunk, Grouse, Clear, and Cub Creeks may be seen in table 3. It is suggested that the early season differential harvest of mature males in the northern lake areas of intense fishing pressure is the cause only for the differences that appear to exist between areas of the lake. Even at Grouse and Chipmunk Creeks, where fish are little affected by the early fishery a significant and constant excess of females in the spawning runs has been measured. This fact indicates that a differential mortality takes place prior to initial spawning. The reason may stem from differential natural mortalities to immature fish in the lake. Barnaby (1944) made note of the excess of females over males in the spawning runs of red salmon in Alaska. He attributed this condition partly to the selective action of the gill net fishery, but suggested that the principal cause underlying this condition lacked explanation. Foerster (1954), noting the same condition for the same species in British Columbia, also indicated that the selective action of the gill net fishery was partially the cause of the differential sex ratio in the spawning runs. He proposed, however, that since the sex ratio of seaward-migrating smolts was 1:1, as Barnaby had observed, the males had become more susceptible to natural mortality during ocean residence.

Weight loss and time spent upstream.—Additional insight into stream survival as it relates to loss in weight and time spent upstream can be obtained from table 15. Evidence from Arnica Creek shows that males remain in the stream for a longer period of time than females.

The males were upstream for an average of 17.3 days over a period of 4 years, while the year-to-year average varied from 12 to 20 days. The female, however, usually remained in the creek for 10 days, although averages for individual years ranged from 6 to 12 days. The average time difference between sexes was 7.3 days. The conclusion is that multiple servicing by males accounts for the difference, which is in keeping with the demands imposed by the unequal sex ratio. Because the average time spent in the stream by Arnica Creek adult females was 6 to 12 days, usually 10, and the reasoning that each must construct 3 redds during that period, it appears reasonable to assume that each female would construct a redd every 2 to 4 days and lay about one-third of her eggs in each redd.

In general, the measurements of weight loss and spawning time tabulated for other streams agree with those obtained at Arnica Creek. There appears to be no obvious relationship between the size of tributary and length of time spent in the stream for spawning. For instance, in 1950 when the sampling of natural spawners at Arnica and Chipmunk Creeks was comparable, an obvious time difference between the larger tributary and the smaller Arnica Creek was not detected. The tendency for males to remain in the stream for longer periods of time than females is evident from all other locations.

Stream-mortality estimates have been made in other trout investigations, and several authors have indirectly shown that survival of adults following spawning is rather low. Belding (1934) correlated the loss of weight associated with the prespawning and spawning period with the low percentage of repeat spawners in mature Atlantic salmon (*Salmo salar*). He inferred that the basic cause of the high mortality was purely physiological. The "natural depletion of the body tissues to the borderline of death by physiological starvation, the development of the sexual organs, and the expenditure of energy" results in a weakened condition and "on its return to the ocean that it is unable to undergo normal recuperation, or it becomes ready prey of its enemies." However, because of the time and distance involved in their spawning migration, Atlantic salmon lost a much higher percentage of body weight than did other salmonoids in other areas. Belding placed the lethal threshold of weight loss at 40 percent and calculated that Atlantic salmon lost from 31 to 44 percent during the reproductive period. These figures may be compared with 16.7 percent and 25.2 percent for male and female rainbow trout (Mottley, 1938), and 18.5 percent and 21.3 percent for male and female Yellowstone Lake cutthroat. However, this relatively small loss of weight by Yellowstone spawners may still be a delayed factor contributing to natural mortality.

Calhoun (1944), working with cutthroat trout (*Salmo clarki henshawi*) in Blue Lake, California, also suggested a close relation between weight loss during spawning and subsequent survival. He said, "Fish returning to the lake after spawning will have small opportunity to build up food reserves to tide them over the winter when the lake is frozen for six months." Calhoun based this supposition on his recognition of the small number of adult cutthroat which spawned a second time. Irving (1953), reporting on the ecology of the cutthroat (*Salmo clarki lewisi*) in Henrys Lake, Idaho, suggested a high rate of spawning and lake mortality on the basis of infrequent trap and fishing returns from marked spawners. While survival and mortality estimates were not presented, he did suggest that spawning loss was the result of injuries, disease, and exhaustion attributable to the activities leading up to and associated with reproductive activities.

Mottley, in his work with rainbow trout in the spawning runs out of Paul Lake, British Columbia, indicated that both the shedding of sex products and utilization of stored food materials were the cause of the loss of weight in males and females (16.7 and 25.2 percent). It was determined that the ovaries of the female constituted only 19 percent of the total body weight, and the testes of the male made up only 5 percent of its total body weight. Allowing for some retention of sex products by both sexes, Mottley estimated that the difference between the total loss of weight and that attributed to the actual loss of sex products, 9 percent for the female and 14 for the male, must be due to the utilization of stored food materials. Even though the female loses a greater percentage of her total body weight during spawning, it is the male who must draw upon a greater store of food reserves in order to survive this period.

Belding (1934) also found that female anadromous Atlantic salmon spawning under natural conditions lost significantly more weight per individual than those from which eggs had been taken by fish culturists. The difference, it must be assumed, can be attributed to the use of stored food materials by natural spawners. He indicated that the development of the eggs and sperm, expenditure of energy and cessation of feeding activities during spawning manifested themselves as stream death (spawning mortality), or as delayed mortality in the ocean as the delayed result of the spawning activities (lake mortality).

Stream mortality and time of migration.—Changes in the rate of stream mortality with the progress of the season were studied at Arnica Creek and viewed in the light of environmental factors which also changed throughout the season. The trout that entered Arnica Creek in early May were usually exposed to cold water and moderate stream flow conditions. These conditions gave way to heavier stream runoff, and the fish in the later parts of migration faced reduced stream flows and higher water temperatures. The trout responded to these changing conditions by changing their times spent in the stream, their distribution within the stream system, and their mortalities.

Table 12 presented the actual enumerations obtained at Arnica Creek traps from 1950 to 1953 and the corrected trap counts and estimated seasonal spawning mortalities derived therefrom. A more detailed analysis of spawning mortality involving

TABLE 16.—*Stream mortality and escapement to the fishery by the tag groups at Arnica Creek, 1950 to 1953*

[Fishing began on May 30 from 1950 to 1952 and on June 15 in 1953]

Time period	Number tagged-up (*NT*)	Number sampled down [*n*(*Td*)]		Number calculated down [*N*(*Td*)]		Group mortality (*sm*)	
		Group	Period	Group	Period	Percentage rate	Number
1950							
May 4–10	0						
May 11–17	0						
May 18–24	53	3	0	12		77	41
May 25–31	89	12	0	50		47	42
June 1–7	233	43	0	178		24	57
June 8–14	275	53	0	220		21	57
June 15–21	100	10	75	42	312	59	59
June 22–28	100	10	19	42	79	59	59
June 29–July 5	25	3	27	12	112	52	13
July 6–12	20	0	3	[1] 10	13	50	10
July 13–19	25	3	2	12	8	52	13
July 20–26	45	7	1	29	4	36	16
July 27–Aug. 2	0	0	7	0	29	0	0
Aug. 3–9	0	0	2	0	8	0	0
Aug. 10–16	0	0	6	0	25	0	0
Aug. 17–23	0	0	1	0	4	0	0
Aug. 24–30	0	0	1	0	4	0	0
Aug. 31–Sept. 6	0	0	0				
Sept. 7–13	0	0	0				
Season total	965	144	144	598	598	38.0	367
1951							
May 4–10	6	1		3		50	3
May 11–17	94	19		53		44	41
May 18–24	112	28		79		29	33
May 25–31	238	73	1	206	3	13	32
June 1–7	181	48	48	135	135	25	46
June 8–14	106	25	86	71	243	33	35
June 15–21	91	12	48	34	135	63	57
June 22–28	114	16	25	45	70	61	69
June 29–July 5	118	13	11	37	31	69	81
July 6–12	65	10	13	28	37	57	37
July 13–19	65	7	15	20	42	69	45
July 20–26	29	4	4	11	11	62	18
July 27–Aug. 2	23	3	7	8	20	65	15
Aug. 3–9	10	2	2	6	6	40	4
Aug. 10–16	0	0	1	0	3	0	0
Aug. 17–23	0	0	0				
Aug. 24–30	0	0	0				
Aug, 31–Sept. 6	0	0	0				
Sept. 7–13	0	0	0				
Season total	1,252	261	261	736	736	41.2	516
1952							
May 4–10	0	0					
May 11–17	0	0					
May 18–24	107	39	11	62	29	42	45
May 25–31	176	47	29	131	78	26	45
June 1–7	261	50	37	210	99	20	51
June 8–14	154	33	25	125	67	19	29
June 15–21	95	31	41	47	110	51	48
June 22–28	20	4	53	13	142	35	7
June 29–July 5	50	8	8	8	21	84	42
July 6–12	29	9	15	9	40	69	20
July 13–19	27	2	2	6	5	78	21
July 20–26	27	5	4	5	11	81	22
July 27–Aug. 2	12	4	5	4	13	67	8
Aug. 3–9	0	0	2		5		
Aug. 10–16	0	0					
Aug. 17–23	0	0					
Aug. 24–30	0	0					
Aug. 31–Sept. 6	0	0					
Sept. 7–13	0	0					
Season total	958	232	232	620	620	35.3	338
1953							
May 4–10	26	13	0	13	0	50	13
May 11–17	63	37	7	37	7	41	26
May 18–24	43	35	5	35	5	19	8
May 25–31	159	98	29	98	29	38	61
June 1–7	147	105	49	105	49	29	42
June 8–14	148	77	96	77	96	48	71
June 15–21	105	51	104	51	104	51	54
June 22–28	88	35	30	35	30	60	53
June 29–July 5	115	33	60	33	60	71	82
July 6–12	68	13	105	13	105	81	55
July 13–19	33	7	13	7	13	79	26
July 20–26	11	1	5	1	5	91	10
July 27–Aug. 2	2	0	2		2	100	2
Aug. 3–9	3	0	0			100	3
Aug. 10–16	5	1	0	1		80	4
Aug. 17–23	0	0	1		1		
Aug. 24–30	0	0	0				
Aug. 31–Sept. 6	3	0	0			100	3
Sept. 7–13	0	0	0				
Season total	1,019	506	506	506	506	50.3	513

[1] Estimated because of a lack of group sample.

tag group mortality rates at Arnica Creek is incorporated into table 16. This table lists the number of upstream tags, *NT*, which bear a constant proportional relation to the numbers of untagged members released upstream in each time period. Figure 4 demonstrates the relation between tag group mortality rates and mean maximum period temperatures at Arnica Creek.

The peak of each spawning run occurred when the mean maximum weekly temperature reached about 55° F. Movement of prespawners (trout approaching sexual maturity prior to spawning, in the lake or in the spawning migration) started at about 45° F. The peak of the run always preceded the period of highest daily water temperatures. Most of the fish for the season had passed upstream before the maximum daily temperature averaged 60° F. Maximum daily temperatures which exceeded 65° F. coincided with the occurrence of large numbers of dead spawners around the trap sites at Arnica and Pelican Creeks. The greatest difference in time between peaks of spawning and temperature occurred at Arnica Creek (5 weeks) and the smallest existed at Chipmunk Creek (1 week). This difference is related to the temperature characteristics of the individual streams, and particularly to temperatures associated with maximum movement of prespawners (53 to 57° F.).

The relation between the tag group spawning mortalities at Arnica Creek and thermal characteristics as reflected by the mean maximum weekly temperatures at the time the fish were enumerated at the upstream trap appears to be an intimate one. Typically, the early run of Arnica spawners suffers a high mortality, followed by a decrease in death rate at the time the peak of the run passes upstream. Thereafter, the group mortality rate increases rapidly, allowing few survivors to escape back into the lake from the terminal portion of the run. The high mortality experienced by the early run fish appears to be independent of the detrimental effects associated with excessive stream temperatures. During the latter half of the spawning runs, a relation between high mortalities and high water temperatures becomes apparent.

Spawners tend to spend less time in the stream as the season progresses. The early run may remain in Arnica as much as 2.0 to 2.5 times as long as the average spawner. Survivors of the last portion of the spawning migration usually spend about half as long in the stream as the average. These calculations are in agreement with the observations of Welsh (1952) and Cope (1956), who noted that the early Arnica Creek spawners characteristically utilized the spawning gravels in the upper portion of the stream. The main segment of the spawning run tended to distribute itself mostly in the middle and lower stream sections, and the late spawners concentrated nest building activities in that stream segment closest to the trap site. Although these late spawners travel the least distance upstream to spawn and spend the least amount of time in the stream itself, it is they who are subjected to the highest stream mortality. Length measurements show that the early portion of the Arnica Creek run is composed of the older and larger fish. The high spawning mortality characteristically incurred by these fish, while not related to adverse temperature conditions, may be attributable to a combination of causes. They may include age, length of time in the stream, and torrential runoff conditions. High water may induce physical injury and fatigue leading to death.

Effects of artificial spawning.—At Pelican Creek in 1950 both natural spawners and artificially stripped adults that had been thrown upstream from the trap after stripping were tagged. While both male and female artificially spawned fish remained in the stream for over a week, no difference between the two groups in time spent upstream was detected. No data are at hand to show why there was no immediate movement of hatchery-processed fish back into the lake. Possibly it may be attributed to the retention of sex products not taken by the fish culturists, or to a period of recuperation from effects induced by handling. A marked difference between natural spawners and artificially spawned fish in average time spent in the stream was noted. Another important feature of this tagging procedure was the determination that artificially spawned fish lost less weight than did the other group.

Marking of 1,135 hatchery-spawned fish and 356 liberated for natural reproduction was also performed at Chipmunk Creek in 1950. The procedure resembled the Pelican Creek operation of the same year. At Chipmunk Creek, however, abundant tag-return data did not become available either from downstream sampling or from the fishery. Therefore, stream mortality for these two groups at Chipmunk Creek could not be reliably estimated. The indications from the ratios of tag returns from both groups collected from dead fish in the stream, the downstream sampling, Molly Island returns, and 1950–52 trap and fishery returns were that hatchery-processed fish had a better survival than did natural spawners.

This condition is in contrast with the results obtained in the identical operation at Pelican Creek. At the northern tributary where hatchery-processed fish were tagged uniformly throughout the spawning migration, their mortality was somewhat higher than that of the group released upstream for natural reproduction. It was calculated that the entire group of Pelican Creek natural spawners incurred stream mortality of 63 percent. The latter portion of this tag group, the late spawners, suffered an estimated 80 percent mortality. The stripped fish were tagged only during the latter part of the run. Their spawning mortality was measured as 94 percent, or 14 percent higher than the corresponding segment of the natural spawner group. Therefore, a combination of the high stream temperatures and multiple handling may well have affected this tag group more adversely than the natural spawners. The apparent reversal of this situation at Chipmunk Creek may best be explained by the predator and temperature relations. The lower water temperatures at Chipmunk Creek (table 2) may not have adversely influenced those fish handled for stripping and tagging purposes to the extent that presumably occurred at Pelican Creek. Because Chipmunk Creek fish released upstream for natural spawning stayed in the stream for a longer time than those released after stripping, their period of vulnerability to predation by pelicans was lengthened and the chance for capture was enhanced. Fish reproducing under natural conditions usually penetrate to shallower parts of the stream during migration and spawning, and thus become more available to the birds. Field observations have shown that adults before and during spawning activities react less to the approach of human beings and other animals than do postspawners. In agreement with the Pelican Creek results was the fact that Chipmunk Creek natural spawners spent from 2 to 3 times as long in the stream as did those that had been stripped.

Alternate and consecutive spawning.—The consequences of spawning may influence the number of postspawners that reproduce a second time. Belding (1934) found that less than 10 percent of Atlantic salmon returned from the sea to spawn a second time. Canadian Department of Fisheries' records were cited in the same report, giving a figure of 2.12 percent for return spawners. Vibert (1950) stated for the same species that a maximum of 3.8 percent of the mature fish he had worked with had spawned previously. Less than 10 percent of the cutthroat trout of Blue Lake, California, reproduced a second time (Calhoun, 1944). Tagging at Yellowstone demonstrated that an average of 1.22 percent of the spawning runs was composed of fish that had spawned before. As a rule, only 7 percent of the postspawners that survived the winter spawned again

TABLE 17.—*Summary of data on alternate and consecutive spawning, from repeat spawners entering traps in spawning streams, by years, 1950–55*

[A=number of tagged fish in the lake; B=number of fish returning; C=percentage of available spawners returning]

Stream and year of tagging	Returns to fish traps																				
	1950			1951			1952			1953			1954			1955			Total		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Arnica Creek:																					
1950	----	----	----	150	51	33.1	37	16	43.2	----	----	----	----	----	----	----	----	----	----	----	----
1951	----	----	----	----	----	----	193	12	6.2	51	9	17.6	----	----	----	----	----	----	----	----	----
1952	----	----	----	----	----	----	----	----	----	33	2	5.4	9	5	55.6	----	----	----	----	----	----
1953	----	----	----	----	----	----	----	----	----	----	----	----	204	20	9.7	53	9	17.0	----	----	----
Total, consecutive	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	580	85	14.7
Total, alternate	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	150	39	26.0
Pelican Creek:																					
1949	138	2	1.5	35	4	11.4	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
1950	----	----	----	108	2	1.9	28	7	25.0	----	----	----	----	----	----	----	----	----	----	----	----
1951	----	----	----	----	----	----	232	5	2.1	57	17	29.8	----	----	----	----	----	----	----	----	----
1952	----	----	----	----	----	----	----	----	----	107	15	14.0	26	11	42.3	----	----	----	----	----	----
1953	----	----	----	----	----	----	----	----	----	----	----	----	227	16	7.0	55	9	16.4	----	----	----
Total, consecutive	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	812	40	4.9
Total, alternate	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	201	48	23.9
Clear Creek:																					
1953	----	----	----	----	----	----	----	----	----	----	----	----	182	2	1.1	47	5	10.6	----	----	----
Total, consecutive	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	182	2	1.1
Total, alternate	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	47	5	10.6
Grouse Creek:																					
1951	----	----	----	----	----	----	239	5	2.1	94	26	27.7	----	----	----	----	----	----	----	----	----
1952	----	----	----	----	----	----	----	----	----	42	6	14.3	11	2	18.2	----	----	----	----	----	----
1953	----	----	----	----	----	----	----	----	----	----	----	----	27	3	10.7	7	1	14.3	----	----	----
Total, consecutive	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	308	14	4.5
Total, alternate	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	112	29	25.9
Chipmunk Creek:																					
1949	317	6	1.8	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----
1950	----	----	----	----	----	----	56	7	12.5	----	----	----	----	----	----	----	----	----	----	----	----
1952	----	----	----	[1]	[1]	[1]	----	----	----	25	3	12.0	7	4	57.1	----	----	----	----	----	----
1953	----	----	----	----	----	----	----	----	----	----	----	----	120	13	11.7	29	5	17.2	----	----	----
Total, consecutive	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	462	22	4.8
Total, alternate	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	92	16	17.4
All streams by tag group:																					
Consecutive	455	8	1.8	258	53	20.5	664	22	3.3	207	26	12.3	760	54	7.2	----	----	----	2,344	163	6.9
Alternate	----	----	----	35	4	11.4	121	30	24.8	202	52	25.7	53	22	41.5	191	29	15.2	602	137	22.8

[1] No trap maintenance in 1951.

the following year as consecutive spawners. Very few fish reproduced two years in succession. An estimated 22.8 percent of those few surviving through their second winter entered the spawning run for the second time. Rarely did an adult cutthroat spawn 3 years in succession.

In 15 cases where the return of spawners from a group of tagged fish was measured, alternate spawners outnumbered consecutive spawners in seven instances (table 17). A total of 300 tagged fish returned to the traps as repeat spawners, of which 137 (45.7 percent) spawned for the second time after a 2-year delay. There was always a greater percentage return of alternate spawners in terms of the number surviving. The consecutive spawners varied from 1.1 to 14.7 percent and averaged 6.9 percent of those available as spawners at the time. On the other hand, the number of alternate spawners ranged from 10.6 to 26.0 percent, averaging 22.8 percent. The returns to the various streams generally fluctuated equally about the mean from year to year.

The significance of this is that adult trout entering a tributary to Yellowstone Lake will seldom spawn a second time. Consequently, the runs are made up largely (always in excess of 90 percent) of fish spawning for the first time.

This alternate-consecutive spawning concept appears to have a physiological basis. The task for postspawners to regain sufficient food stores and an adequate physical condition to survive the winter is difficult. Presumably, during the winter they are almost completely dependent on an intrinsic source of food. In the Yellowstone environment, few postspawners would be physically able to develop sexually as consecutive spawners during this period of time. Even the following year

TABLE 18.—*Average mortalities and survivals in Arnica Creek spawning run of 1,000 spawners covering a period of 2 years, based on the history of spawning runs from 1950 to 1953*

Phase of life history	Mortalities (number of fish)			Survivals (number of fish)	Chronology	Age predominance
	Stream	Fishing	Lake			
Enter spawning run				1,000	May–June	III–IV–V
Spawning mortality	481					
Postspawners available to fishery				519		
Fishing mortality		60			June–Sept	
Natural mortality			319		June–May	
Adults surviving winter				140	May	IV–V–VI
Consecutive spawners				10	May–June	
Spawning mortality	5					
Alternate (non) spawners				130		
Adults available to fishery				135		
Fishing mortality		16			June–Sept	
Natural mortality			83		June–May	
Adults surviving winter				36	May	V–VI–VII
Accountability, in numbers of fish	486	76	402	36		
Percentage of original spawners	48.6	7.6	40.2	3.6		

finds only about 75 to 80 percent of the surviving fish capable of reproducing again.

The potential of repeat spawners is depicted in table 18. Beginning with a spawning run of 1,000 fish, average mortalities derived from stream locations, to be discussed later, are imposed. These data demonstrate that only 10 fish will function as consecutive spawners, and the potential alternate spawners number 42. Thus, the maximum number of fish spawning a second time, assuming that all of the potential alternate spawners will return to spawn, would not exceed 5.2 percent of the original 1,000 adults. The expected return of repeat spawners, however, would be about 2.4 percent (one-third of 5.2 percent, those available to spawn a second time).

The white pelican and the cutthroat.—The white pelican colony at Molly Island in the southeast arm of Yellowstone Lake is important in relation to trout in this drainage. It was "conservatively" estimated in 1922 that around 300,000 cutthroat trout were consumed annually by these piscivorous birds (Ward, 1922).[6] This figure may apply from year to year, since the number of pelicans breeding in Yellowstone Park seems to have remained rather stable for many years.

Grinnell (1876) reported pelicans to be "very abundant" at the time of his visit in 1875. Skinner (1925) observed from 500 to 700 birds visiting the rookery annually from 1898 to 1917. In the summer of 1922, Ward estimated about 500 to 600 adults and 200 fledglings. Bird banding teams of the National Park Service from 1949 to 1951 estimated from 450 to 500 adult pelicans frequenting the islands annually. Biologists of the Fish and Wildlife Service counted more than 400 adult birds in 1953.

From 1924 to 1931 the pelican population was controlled by reduction in the number of eggs and adults. After control was terminated, Thompson (1932) reported only 300 adults and 100 young. Since 1932, the white pelicans have received complete protection. The public is forbidden to land or approach the two Molly Islands in the Southeast Arm. This breeding colony, therefore, is legally protected from exploitation, afforded protection from man by restricting of boat travel to the area, sheltered from mammal predation by virtue of the rookery's separation from the mainland, furnished a ready access from the nesting sites to the water for the young pelicans, and surrounded by an abundant supply of fish which become particularly available during the spawning migrations into tributaries of Yellowstone Lake. These conditions favor survival of immature birds, and the perpetuation of the colony.

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[6] Ward, H. B. The pelicans of Yellowstone Lake. Manuscript prepared for U.S. Bureau of Fisheries in cooperation with U.S. National Park Service, 1922. 20 pp.

The first group of adult pelicans may arrive at Yellowstone Lake as early as May 1, and the rest of the colony will arrive in groups later in the month. The early arrivals begin their fishing activities along the Yellowstone River. Later, when the ice begins to leave the lake, the birds retire to the Molly Islands and begin breeding activities.

Two, occasionally one or three, eggs per nest are laid in May or early June. The incubation period is three or four weeks. The simply constructed nest are guarded by both parents, one at a time, at least during the incubation of the eggs and early rearing of the young. While one parent is away foraging for fish, the other parent remains on the nest. The morning flight has been observed to leave the Yellowstone rookery around daybreak and return around noon. The other parent then spends the afternoon fishing, returning to the nesting area around dusk. Feeding may continue into the night, as observed by Low, Kay, and Rasmussen (1950). Newly hatched pelicans feed upon the regurgitated fish offered by their parents. Growth is extremely rapid. The adult birds must not only satisfy their own enormous appetites, but those of the growing offspring. Studies at Molly Island indicate a third to a half of the total egg production survive to the fall as juvenile birds.

The feeding and nesting times of the adult pelicans at Yellowstone coincide precisely with the time that the mature cutthroat enter the spawning tributaries and become available to pelicans. Both parent birds have a limited time for foraging before returning to the rookery when the eggs are incubated and the young birds protected. This occurs when the trout are spawning. Hall (1925) appropriately suggested that "the white pelican eats the food most easily secured." During the 50 or 60 days of the movement of trout into tributaries of Yellowstone Lake, the pelicans prey almost exclusively upon this available and vulnerable fish. This view agrees with the field observations of Ward, who indicated that the white pelicans of Molly Island "were most abundant on streams where the spawning runs were the largest." Ward also estimated that Yellowstone trout made up 98 to 100 percent of the pelican's diet, particularly during the time of trout spawning.

Later in the season when the immature pelicans require less protection, both parent birds can be absent from the rookery for longer periods of time. The surviving trout have returned from the streams to the lake, and are less vulnerable to capture; consequently, the pelicans may feed in waters some distance removed from the Southeast Arm of Yellowstone Lake. Then, too, more time and effort may be devoted to obtaining mature and immature trout and suckers from shoal areas of the lake. Large flights of adult pelicans have been noticed leaving Yellowstone Lake in the late summer, presumably in a search for fishing grounds elsewhere (Skinner, 1925). Pelicans have been known to make daily flights of 100 to 150 miles to obtain their fish (Low, Kay, and Rasmussen, 1950).

The brown pelican (*Pelecanus occidentalis*) characteristically captures fish by diving from a considerable height directly onto the water. The white pelican fishes while on the surface of the water and obtains prey by rapidly plunging its head into the water. Pelicans are patient and efficient predators both in the streams and in the lake. Observers have seen them team up in a line and herd fish into shallow areas of Yellowstone Lake where capture was easier.

To estimate total mortality of trout from pelican predation, it is necessary to know at what rate fish are captured. Information on consumption rate and growth of young has been provided by Hall (1925) for white pelicans of Pyramid Lake, Nevada. The fledglings ate about half a pound of fish a day 10 days after hatching.

TABLE 19.—*Food consumption of the white pelican at Yellowstone Lake*

[In pounds of fish]

Number of pelicans	During a feeding day			During a 55-day spawning cycle			During a 100-day lake residence		
	Hall	Ward	Average	Hall	Ward	Average	Hall	Ward	Average
1	4	11	7.5	200	605	412	400	1,100	750
100	400	1,100	750	22,000	60,500	41,200	40,000	110,000	75,000
200	800	2,200	1,500	44,000	121,000	82,400	80,000	220,000	150,000
300	1,200	3,300	2,250	66,000	181,500	123,600	120,000	330,000	225,000
400	1,600	4,400	3,000	88,000	242,000	164,800	160,000	440,000	300,000
500	2,000	5,500	3,750	110,000	302,500	206,000	200,000	550,000	375,000
600	2,400	6,600	4,500	132,000	363,000	247,200	240,000	660,000	450,000
700	2,800	7,700	5,250	154,000	423,500	288,400	280,000	770,000	525,000

A month after hatching, their consumption had increased to 2 pounds of fish each day. At this time, the down-covered birds weighed 6 to 7 pounds. Fifteen days later, 55 days after hatching, they had attained weights of 10 to 12 pounds, and their daily food consumption had doubled. The first flight trials occurred around the 63d day, at which time each surviving offspring had accounted for a total of 150 pounds of fish. They then weighed 12 to 13 pounds and exhibited appetites characteristic of adult birds.

Hall's estimate of the consumption rate for adult birds is 4 pounds of fish per day. He stated, however, that "pelicans frequently fish until the esophagus up to the mouth is filled and greatly distended." Bent (1922) often observed a captive white pelican consuming 6 to 8 pounds of food at a single meal. In a tributary to Yellowstone Lake, Ward witnessed a rate of capture of 2 adult fish per hour. Fishing at least 6 hours per day during the first 2 months at the rookery, each parent bird would take about 12 fish (10 to 12 pounds) per day. A portion of the food taken, of course, would be fed to the fledglings. From these data, Ward's mortality estimate of 300,000 adult trout was computed only through the 50-day spawning cycle of the fish. The rest of the time spent fishing by the pelicans was not included; hence, his estimate was considered to be minimal. The utilization rates by white pelicans at various population levels and using consumption rates of Hall, Ward, and a mean rate of 7.5 pounds per day have been consolidated in table 19. Consumption is given in pounds, and conversion to fish numbers is accomplished by using a factor of 1.14, a figure derived from average weights of adult fish in the population.

Immature pelicans are fed by their parents for about 75 days at Molly Island; that is, to the time of southward departure in early September. Since each fledgling consumes approximately 150 pounds of fish during the first 63 days and with a low daily consumption rate of 4 pounds thereafter, each bird-of-the-year requires almost 200 pounds of food to the time of migration. Assuming these fish to be adult cutthroat trout from Yellowstone Lake, then the 200 young reared at Molly Island in 1953 alone accounted for at least 40,000 trout.

Yellowstone Lake and its tributaries are favorable for pelican feeding. Not only are cutthroat trout the principal inhabitants of the lake, but they become highly vulnerable to pelicans and other predators during temporary stream residence as spawners. In a single day a population of 500 adult pelicans may capture 3,750 pounds of fish (4,275 adult trout). During the 55-day spawning cycle of the trout, the same number of birds can account for 234,840 fish. Even Hall's minimal consumption rate extended over this 2-month period totals 125,400 trout. Regardless of which consumption rate is most appropriate, there is little doubt that the white pelican is the principal animal predator on adult cutthroat trout at Yellowstone

TABLE 20.—*Record of tag recoveries, 1949 to 1954*

[Numbers in parentheses denote returns from stream fishing other than Yellowstone River]

Tag group	Number of fish tagged	Numbers of tags recovered												
		1949		1950		1951		1952		1953		1954		
		Fishery	Molly Island	Fishery	Molly Island	Fishery	Molly Island	Fishery	Molly Island	Fishery	Molly Island	Fishery	Molly Island	Total
1949:														
Chipmunk Creek	649	11	28	35	0	0	0	0	0	0	0	0	0	74
Pelican Creek	750	27(4)	33	14(2)	0	0	0	0	0	0	0	0	0	80
1950:														
Chipmunk Creek	1,491	--------	----	19	30	12	0	1	0	0	0	0	0	62
Pelican Creek	2,200	--------	----	54(15)	20	9(2)	0	1	0	0	0	1	0	102
Arnica Creek	965	--------	----	44	0	17	0	2	0	0	0	0	0	63
1951:														
Grouse Creek	1,196	--------	----	--------	----	29	11	15	0	0	0	0	0	55
Pelican Creek	1,295	--------	----	--------	----	75(20)	2	28(3)	0	1	0	0	0	129
Arnica Creek	1,252	--------	----	--------	----	82	3	12	0	0	0	0	0	97
Yellowstone River	399	--------	----	--------	----	89	1	22	0	7	0	1	0	120
1952:														
Chipmunk Creek:														
Up	408	--------	----	--------	----	--------	----	2	27	0	0	0(2)	0	31
Down	125	--------	----	--------	----	--------	----	9	0	2	0	0	0	11
Grouse Creek:														
Up	492	--------	----	--------	----	--------	----	3	49	2	0	0	0	54
Down	202	--------	----	--------	----	--------	----	2	0	1	0	6	0	9
Pelican Creek:														
Up	744	--------	----	--------	----	--------	----	72(11)	5	13(1)	0	7	0	109
Down	167	--------	----	--------	----	--------	----	20(2)	1	1	0	0	0	24
Arnica Creek	958	--------	----	--------	----	--------	----	40	9	3	0	7	0	59
Yellowstone River	400	--------	----	--------	----	--------	----	71	0	24	0	1	0	96
Trap net	454	--------	----	--------	----	--------	----	63	0	20	0	2	0	85
Southeast arm	265	--------	----	--------	----	--------	----	1	0	0	0	0	0	1
1953:														
Chipmunk Creek:														
Up	440	--------	----	--------	----	--------	----	--------	----	11	22	7	0	40
Down	112	--------	----	--------	----	--------	----	--------	----	5	1	1	0	7
Grouse Creek:														
Up	431	--------	----	--------	----	--------	----	--------	----	5	28	2	0	35
Down	172	--------	----	--------	----	--------	----	--------	----	7	0	0	0	7
Pelican Creek:														
Up	1,246	--------	----	--------	----	--------	----	--------	----	114(7)	4	30(1)	0	156
Down	77	--------	----	--------	----	--------	----	--------	----	2	1	1	0	4
Arnica Creek:														
Up	1,019	--------	----	--------	----	--------	----	--------	----	40	3	12	0	55
Down	353	--------	----	--------	----	--------	----	--------	----	29	3	5	0	37
Yellowstone River	379	--------	----	--------	----	--------	----	--------	----	78	1	9	0	88
Trap net	275	--------	----	--------	----	--------	----	--------	----	68	0	11	0	79
Clear Creek:														
Up	497	--------	----	--------	----	--------	----	--------	----	24(5)	1	11	0	41
Down	595	--------	----	--------	----	--------	----	--------	----	19	1	4	0	24
Total	20,008	38(4)	61	166(17)	50	313(22)	17	364(16)	91	476(13)	65	108(3)	0	1,834

Lake. Animal predation probably has always accounted for more trout than has the sport fishery.

The return of fish tags from fecal deposits on Molly Island gives an indication of the area and relative impact of pelican activity, although not all tags are returned to the rookery (see table 20).

These data demonstrate that pelicans concentrate their fishing largely in the southern part of the lake. Chipmunk and Grouse Creeks provided greater numbers of recovered tags than did other tagging locations, as illustrated in figure 5. Field observations over a period of several years have detected large concentrations of these birds to be associated with the spawning runs of the larger tributaries in the South and Southeast Arms. The large number of Pelican Creek tags recovered from the rookery in 1949 may be related to the unusually large spawning run that entered the stream that year.

All Arnica Creek tags that were recovered on the Molly Islands came from fish that had been checked through the downstream trap, suggesting that lake predation is probably characteristic for postspawners from stream areas farthest removed from Molly Island. In general,

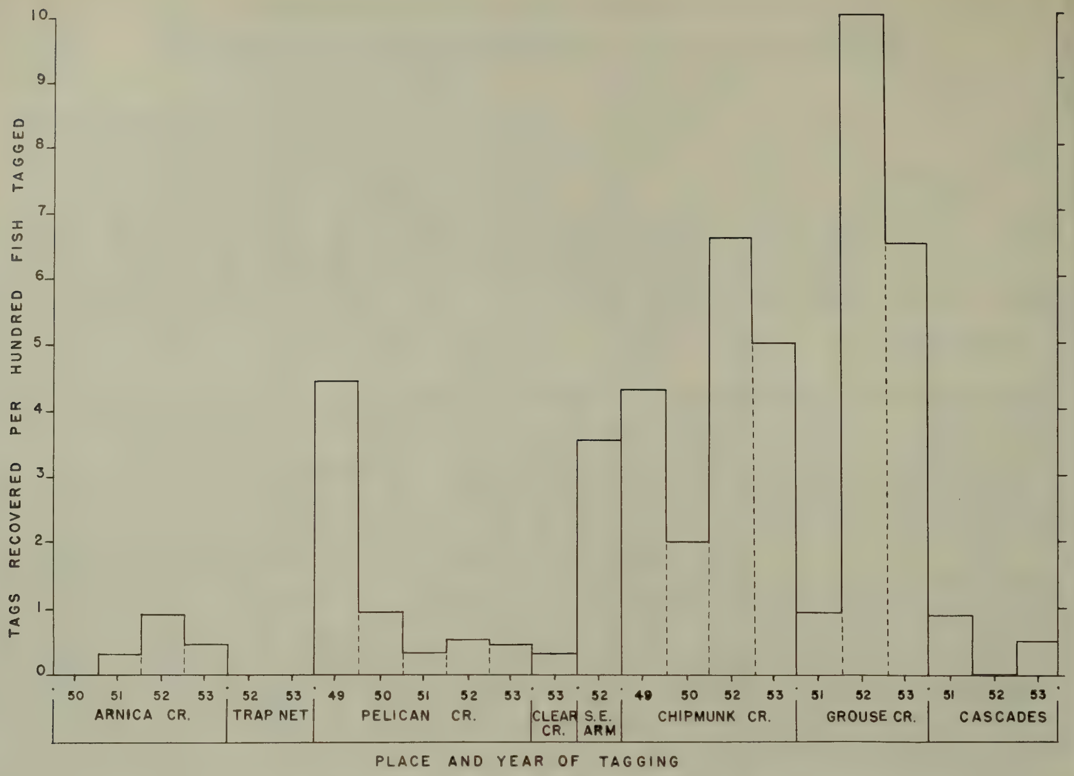

FIGURE 5.—Results of tag collections from the pelican rookery at Molly Island, 1949–53.

stream predation predominates in the southern half of Yellowstone Lake, and especially in the larger tributaries.

Increased vulnerability of fish with bright-colored tags to pelican predation, as compared with those without tags, has been considered in these studies. Of the 2,203 tags applied at Pelican Creek in 1950, 1,878 were yellow and 325 were dull green. The subsequent returns from Molly Island totaled 20 and consisted of 17 yellow and 3 green tags. Thus, where tags of two colors of different degrees of brightness were applied at a single location, the two colors were apparently taken by pelicans at about the same rate. No estimate of increased susceptibility to capture due to the presence or absence of a tag, or to any difference between bright-yellow and dull-green tags, is available.

The recovery of fish tags from the white pelican rookery at Molly Island, and ecological studies on this bird and the fish population, bear out the following conclusions: (1) The principal animal predator of the trout of Yellowstone Lake is the pelican, which draws its toll principally from mature fish in May, June, and July; (2) total natural mortality inflicted by pelicans presently exceeds the mortality resulting from the sport fishery; (3) the spawning runs of the southern part of the lake are most seriously affected where these birds forage principally at the mouths of larger tributaries and on the spawning gravels; (4) fish in the northern and West Thumb sections of Yellowstone Lake are relatively little affected, and stream fishing by pelicans there is more common than is lake fishing; (5) although pelicans may frequent the upper Yellowstone River and its tributaries in large numbers, the river below fishing bridge is visited by relatively small numbers of birds throughout the season; and (6) the take of fish by the pelicans appears to be a combined function of availability of fish, proximity of fishing areas to the rookery, size of stream or depth of lake, and amount of human activity in the area.

Summary of mortality associated with spawning.—The conditions that influence spawning mortality of cutthroat trout may be summarized as follows:

1. Of adult fish migrating into tributaries of Yellowstone Lake, more than 90 percent are initial spawners between 3 and 5 years of age.

2. Time spent in the stream by both sexes varies during the spawning run, and appears to depend on aquatic conditions. Males usually remain upstream longer than females. This is at least partly due to the numerical dominance of females in the runs, necessitating multiple servicing by males.

3. The total loss of body weight (sex products and stored fat and muscle) by females exceeds that by males, but the males lose a greater percentage of fat and muscle than the females, probably because of greater activity and a longer time in the stream. Under adverse conditions the mortality of male spawners may be higher than that of females. An equal stream mortality for both sexes can be expected under normal conditions.

4. The rate of stream mortality varies widely from stream to stream in any given year, and in the same stream from year to year.

5. Sources of natural mortality in the stream differed from area to area. The aquatic environment was warmest at Pelican and Arnica Creeks, and the rate of spawning mortality was highest when the stream temperature reached a peak. At Arnica Creek, crowding due to the relatively large spawning run for the stream size made spawners more vulnerable to predation by bears. At Pelican Creek, where the distance to the spawning gravels was much greater, pollution from geyser basins and hot mineral springs materially contributed to greater year-to-year fluctuations in stream mortality. Grouse and Chipmunk Creeks support a more favorable stream condition. Here, deaths in the stream are largely attributable to predation by pelicans. The proximity of both streams to the rookery at Molly Island. the limited human activity in the area, and the sizes of the tributaries and spawning runs give the birds ready access to the spawning fish. At all locations, stream predation by pelicans, bears, and other fish-eating animals account for an unknown number of spawners before, during, and after egg deposition. In the Fishing Bridge spawning area, the fishermen hold the same relation to the mature fish. However, even if activities of natural and human predators were nonexistent, and if aquatic conditions were ideal, a significant spawning mortality should nevertheless occur. The tagging operation at Clear Creek, a stream which most closely approaches such an ideal condition, supports this view.

6. Natural mortality in the stream itself, subsequent survival, and a seldom-realized second spawning are functions of the physical condition and activities of the adults before and after spawning. During the year following spawning, natural mortality accounts for about 60 percent of those that survive and return to the lake. These deaths are assumed to be greatest during the ensuing winter. The inability of postspawners to regain an adequate physical condition during the summer's feeding to meet the biological demands of the severe winter environment that follows may largely determine natural mortality in the lake.

7. The conclusion that few trout spawn in consecutive years has a sound physiological basis. Postspawners are seldom in a condition to mature sex products for the next year and at the same time maintain themselves through the winter. Hence, the following summer must also be used for recuperation. Some are then able to function the second spring as alternate spawners. However, natural mortality through the postspawning period reduces

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the numbers of surviving adults so greatly that few are available to spawn again.

Fishing mortality of postspawners

Efficiency of tag recoveries in 1953.—A roadblock interview with fishermen was made to obtain data pertaining to tag-recovery efficiency. Table 21 shows that of the 35 tags located in the interviews, it was decided that 4 would not have been turned in. Two of the six tags that had not been turned in before the interviews were considered to be tags that would have been returned. The remainder of the contacted tags (29) had already been checked in. This return of 31 out of 35 tags suggested that 11.4 percent of the tags from fish actually caught by the fishermen might not be returned.

Lake fishing.—Studies on fishing mortality in Yellowstone Lake were carried on in several ways, with tagging as a tool to relate numbers of fish caught by fishermen to numbers previously released from traps in streams or into the lake. Fish released from trap nets into the lake became immediately available to fishermen, but those trapped in streams often were delayed in their entrance into the catch. Most fish taken in lake traps were intercepted on their way to spawning areas near Fishing Bridge; those released downstream from stream traps had completed their spawning. The methods of analysis of contributions to the catch from these populations varied according to the circumstances at the several locations.

TABLE 21.—*Tag-return efficiency, as determined from the road-block interview, 1953*

Date		Number of persons interviewed	Number who fished	Number of tags			
				Total	Turned in	Would have been turned in	Not turned in
June	21	2,687	280	0	0	0	0
	28	1,581	345	2	2	0	0
July	5	2,094	371	4	4	0	0
	12	1,757	354	1	1	0	0
	19	4,007	686	12	7	2	3
	26	1,791	363	7	6	0	1
Aug.	2	2,534	194	1	1	0	0
	9	1,906	283	4	4	0	0
	16	3,613	436	3	3	0	0
	23	1,149	127	0	0	0	0
	30	1,282	116	1	1	0	0
Sept.	6	1,630	132	0	0	0	0
	Total	26,031	3,687	35	29	2	4

The methods employed in this report differ in detail and application, but not in principal, from those of Graham (1938). The effect of the fishery on a stock of marked fish may be analyzed from an instantaneous or rate-of-fishing standpoint and as a seasonal rate, termed fishing mortality.

The rate of fishing uses the tag returns for each tagging location during a time period and expresses them as a percentage of the cumulative availability of that particular group of tagged fish. Since tagging and escapement to the fishery occurred before and during the period of most intense fishing, the number of tagged fish liberated into the lake was accumulated (less prior tag returns) to the end of each time period (cumulative availability). Analysis was based on the accumulation of nonaccountable tags throughout the season. No correction was made for concurrent natural mortality throughout the fishing period. The rate of fishing can be assumed to approximate the degree of exploitation affecting that population of adult fish at any given time.

Fishing rate is also given as period fishing returns or rate of tag return. This measure presents the results from limited data as comparable with data from more extensive experiments where the rate of fishing analysis was used. Period returns expresses the number of tags returned within a weekly time period as a percentage of the total for the season for each group of tagged fish.

Two independent measures of seasonal fishing mortality are used. One, tag group fishing mortality, is computed from the total tag returns per season in relation to the total number exposed to the fishery. A tag group includes the number of fish tagged during a time period. Therefore, only the survivors of each tag group become exposed to the fishing pressure. This measure of fishing depends on precise information about stream mortality rates, and was used only for Arnica Creek from 1950 to 1953. The period of stream habitation and the time of tagging, exposure to the fishery, and general physical condition of postspawners varies for each tag group throughout the season. These variables determine when and how many postspawners enter the lake.

The second measure of seasonal fishing mortality, time group fishing mortality, deals with the total number of tagged fish (time group) checked downstream into the lake during a time period. This fishing mortality expresses the number of tagged fish taken by fishermen during the season as percentage of the appropriate time group. A time group originates by a complete count of a downstream run containing tagged fish (Arnica Creek, 1953). If tagging of postspawners takes place, the number marked each week is also a time group (Arnica Creek, 1953; Pelican Creek, 1952–53; Chipmunk and Grouse Creeks, 1952–53; Clear Creek, 1953).

The principle difference between these two methods lies in group identification. Fishing mortality for all marked fish averaged through the entire fishing season was used at most stream locations because tagging procedures were of a less precise nature than those used at Arnica Creek.

The tagging operations at the Cascades and trap net could not differentiate tag and time groups; consequently, they are identical.

The sparse fishery tag returns from Chipmunk and Grouse Creeks necessitated the combining of measurements of fishing intensity for the two locations in 1952 and 1953. This procedure was considered valid because the streams are located only a few miles apart, and the characteristics of their spawning runs and the subsequent tag returns from the fishery were similar in pattern.

Arnica Creek from 1950 through 1953 provided the most complete mortality data for any stream location. Stream mortality data have been summarized previously both in text and appendix tables. Measurements of fishing intensity for Arnica Creek are summarized in table 22. The complete data from which this summary was prepared appear in appendix tables 1 and 2.

TABLE 22.—*Measurements of fishing intensity on fish tagged at Arnica Creek, 1950–53*

Time periods	Tag group fishing mortality (number of fish)				Time group fishing mortality (number of fish) 1953	Rate of fishing (percent of fish available)				Period returns (number of fish)			
	1950	1951	1952	1953		1950	1951	1952	1953	1950	1951	1952	1953
May 4–10	------	0	------	15	------	------	------	------	------	------	------	------	------
May 11–17	------	23	------	11	14	------	------	------	------	------	------	------	------
May 18–24	33	13	11	6	0	------	------	------	------	------	------	------	------
May 25–31	6	13	10	11	16	------	------	------	------	------	------	------	------
June 1–7	6	6	6	10	12	------	0.0	0.0	------	------	------	------	------
June 8–14	4	10	4	4	10	------	1.3	1.8	------	------	6	13	------
June 15–21	10	15	2	12	13	0.0	1.6	1.6	1.9	0	10	15	13
June 22–28	20	16	0	3	6	0.5	2.1	1.0	0.8	5	15	13	6
June 29–July 5	17	8	0	0	9	2.4	1.7	1.3	1.5	27	12	18	13
July 6–12	20	7	11	0	1	1.4	1.1	1.4	0.9	16	9	20	10
July 13–19	17	5	17	0	0	1.2	1.8	0.4	1.0	14	15	5	12
July 20–26	0	0	0	0	0	1.0	1.7	0.4	1.1	11	13	5	13
July 27–Aug. 2	------	0	0	0	0	0.6	0.3	0.2	1.4	7	2	2	16
Aug. 3–9	------	0	------	------	0	0.6	0.9	0.0	1.0	7	7	------	12
Aug. 10–16	------	------	------	0	------	0.5	0.6	0.5	0.3	7	5	8	3
Aug. 17–23	------	------	------	------	0	0.2	0.3	0.2	0.3	2	2	2	3
Aug. 24–30	------	------	------	------	0	0.2	0.3	0.0	0.0	2	2	------	------
Aug. 31–Sept. 6	------	------	------	------	0	0.2	0.2	0.0	0.0	2	1	------	------
Sept. 7–13	------	------	------	------	------	0.0	0.0	0.0	0.0	------	------	------	------
Total number	------	------	------	------	------	------	------	------	------	44	82	40	69
Average (percent)	7.4	11.1	6.5	7.9	8.0	------	------	------	------	------	------	------	------

The run of adult fish into Arnica Creek usually begins early in May and reaches a peak later in the month or in early June. During 4 years of investigation, more than two-thirds of the Arnica postspawners entered the lake each year between June 1 and July 12. Between 7.3 and 12.4 percent of the tagged fish were later caught by the fishermen. Fishing mortality for individual tag groups ranged as high as 33 percent. The tag groups entering the stream earliest are usually more efficiently harvested than are those tag groups from the latter part of the spawning run. Time group fishing mortalities in 1953 produced rates in keeping with those estimated for tag groups.

Arnica Creek fish were most efficiently harvested by fishermen after the first week in June through the middle of July, as indicated by the rate-of-fishing estimates. As an average, the rate of fishing equaled or exceeded 1.0 for a period of 5 or 6 weeks each season. After the middle of July, the fishery for Arnica Creek postspawners declined markedly. Year-to-year variations in distribution of fishing rates within the season are evident, although the success with which they are harvested for an entire season appears to be quite stable.

Period returns demonstrated that the catch of Arnica Creek fish was well distributed thoughout the fishing season. Over half of the tagged fish were taken from the last week in June through the end of July. In 1950, the period of June 29 through July 5 was the week of most successful fishing (rate of fishing, 2.4) and the week of the greatest number of tag returns (27 percent of the total returns at that time). However, in 1952 the most efficient catch took place from June 8 to June 14, and the greatest number of postspawners was taken during the period from July 6 to July 12.

Usable rates of fishing were also obtained from Pelican Creek for 1952 and from Chipmunk and Grouse Creeks combined for 1952 and for 1953 (table 23). The complete information pertaining to this summary can be studied in appendix tables 3 and 4. The most efficient fishing for Pelican Creek fish took place from July 20 to July 26. The rate of fishing was 3.2 and compares with maximum values of 1.8 for Arnica Creek and 1.7 for Chipmunk and Grouse Creeks for the same year. Pelican Creek sustained a fishing rate of 1 or greater over a period of 5 weeks, as did Arnica Creek the same year. Chipmunk and Grouse Creeks supported comparable rates for only 2 weeks in 1952 and 1 week in 1953.

TABLE 23.—*Rates of fishing obtained from tagging at Pelican Creek for 1952 and at Chipmunk and Grouse Creeks for 1952 and 1953*

[Expressed as number of tagged fish captured per 100 available during each time period]

Time period	Rate of fishing in—		
	Pelican Creek 1952	Chipmunk and Grouse Creeks 1952	Chipmunk and Grouse Creeks 1953
June 15–21	0.0	0.0	-----------
June 22–28	0.0	0.0	-----------
June 29–July 5	0.6	0.0	0.0
July 6–12	3.0	1.3	0.0
July 13–19	1.9	0.0	0.0
July 20–26	3.2	1.7	0.0
July 27–Aug. 2	1.3	0.6	0.7
Aug. 3–9	0.7	0.9	1.8
Aug. 10–16	2.0	0.3	0.7
Aug. 17–23	0.0	0.0	0.7
Aug. 24–30	0.0	0.0	0.0
Aug. 31–Sept. 6	0.0	0.0	0.4
Sept. 7–13	0.0	0.0	0.0

The only measurement of fishing intensity furnishing a comparison between all tagging operations throughout the program is the period fishing returns. A summary of first-year returns (tagged fish caught the same year as tagged) for each tagging location averaged for all years is seen in table 24. Supplemental data pertaining to period tag returns, which includes information concerning second-year tag returns (tagged fish caught the year after tagging), may be found in appendix tables 5 and 6. A general resume of all first and second year tag returns is included in appendix table 7.

Considering all areas combined, 70 percent of all available tagged fish were taken by fishermen in the month of July. More marked fish from the Cascades and

TABLE 24.—*Summary of first-year period fishing returns for various years from all tagging locations*

[Expressed as percentage of total returns for each stream]

Time period	Period tag returns from—						All areas
	Arnica Creek 1950–53	Pelican Creek 1949–53	Clear Creek 1953	Chipmunk and Grouse Creeks 1949–53	Cascades 1951–53	Trap net 1952–53	
June 1–7	----	0.3	----	----	1	----	0.4
June 8–14	4	2	----	1	0.4	----	2
June 15–21	10	2	2	----	3	----	3
June 22–28	10	3	2	1	6	6	5
June 29–July 5	16	10	2	2	26	29	16
July 6–12	12	13	9	4	23	26	16
July 13–19	12	10	9	7	18	22	13
July 20–26	11	21	19	15	12	8	15
July 27–Aug. 2	7	14	16	18	5	5	10
Aug. 3–9	7	10	23	16	2	2	8
Aug. 10–16	5	7	----	17	1	1	5
Aug. 17–23	3	4	14	12	1	----	4
Aug. 24–30	1	4	2	2	----	----	2
Aug. 31–Sept. 6	1	----	----	4	0.4	----	1
Sept. 7–13	----	----	----	3	.4	----	.4
Total number of returns	235	364	43	103	238	129	1,112

trap net were caught during the opening week of the river fishery (July 1) than in any other week. Clear, Chipmunk, and Grouse Creeks were characteristically late (July or early August) in contributing fish to the creel.

The effects of fishing on tagged fish from the trap net (table 25) reveal some important contrasts with stream locations. Fishing mortality was several times greater than those estimated for tributary post-spawners. All tag (or time) groups for the trap net sustained heavier fishing mortalities. This was especially true for the tag groups marked during the height of the Fishing Bridge area fishery, from June 22 to July 15 in 1952 and 1953. The rates of fishing demonstrate that although the fishery for trap net fish was of short duration, the success was very high. A short time between tagging and capture is indicated by the period returns. An average of 79 percent of the trap net fish taken by anglers were caught between June 29 and July 19. Seasonal fishing mortality sustained by trap net fish averaged 21 percent. During the week when fishing was most successful, from 7.9 to 14.1 adult trout per 100 available were caught by anglers. Additional information relating to the trap net tagging operation is included in appendix tables 8 and 9.

TABLE 25.—*Measurements of fishing intensity on fish from the trap net, 1952 and 1953*

Time period	Tag/time group fishing mortality (number of fish)		Rate of fishing (percent of fish available)		Period returns (number of fish)	
	1952	1953	1952	1953	1952	1953
June 15–21	0	33	----	0.0	----	----
June 22–28	25	31	4.5	2.9	8	4
June 29–July 5	28	28	7.1	14.1	18	40
July 6–12	21	14	7.9	7.9	28	25
July 13–19	2	13	5.3	6.5	25	21
July 20–26	8	10	2.0	1.9	10	6
July 27–Aug. 2	----	----	1.3	1.0	7	3
Aug. 3–9	----	----	.7	.5	3	1
Aug. 10–16	----	----	.3	.0	2	----
Total number	----	----	----	----	61	68
Average (percent)	17.3	24.7	----	----	----	----

In 1952, 101 fish tagged at the trap net were considered to be immature, that is, were under 300 mm. in total length and did not appear to be gravid. Only two tags from this group were returned by fishermen in 1952. The fishing mortality of 2.0 percent for immature fish, when compared with 17.3 percent for the adult trout, indicated that availabilities of both tag groups were not comparable or that the fishery was selective. Only 5 tags from this immature fish group were taken in 1953 and none in 1954. Considering the mortality estimates for trap net fish tagged in 1953, it was obvious that an extrinsic mortality was imposed on the smaller fish in 1952. Full recruitment into the fishery was contemplated in 1953 and 1954, but it did not occur. Possibly the the handling and tagging of smaller fish was detrimental and resulted in delayed mortality after being released. This group was separated from the adult fish for purposes of analysis.

Fishing-intensity measurements for fish tagged at the Cascades of the Yellowstone River (table 26) show results similar to those obtained from the trap net operation. Estimates of fishing mortality for "lake" and "river" fish indicated comparable magnitudes. The first week in July was also the time when river fish were most efficiently taken by anglers. However, the fishermen began catching Cascades fish several weeks before the trap net fish, because of the earlier spawning run in the river. The harvest was also more prolonged, as demonstrated by the period returns and fishing rates. Adult fish from the Cascades and trap net were caught in greatest numbers when the river and Fishing Bridge opened to the anglers. Appendix tables 10 and 11 contain additional information pertaining to the Cascades tagging operation.

The actual return of tags from angler-caught fish is estimated to be about 88.6 percent of the actual take of tagged fish, based on a program of interviewing fishermen in 1953. Corrected mortalities for the season (table 27) did not modify the results on a relative basis. However, this refinement is valuable when used in absolute computations. For instance, when computing the contribution of postspawners to the lake fishery (table 28), the use of corrected fishing mortalities gives a more realistic measure of value.

TABLE 26.—*Measurements of fishing intensity on fish from the Cascades, 1951 to 1953*

Time period	Tag/time-group fishing mortality (number of fish)			Rate of fishing (percent of fish available)			Period returns (number of fish)		
	1951	1952	1953	1951	1952	1953	1951	1952	1953
May 25–31	----	18	----	----	----	----	----	----	----
June 1–7	----	19	0	----	2.7	----	----	4	----
June 8–14	16	17	17	0.0	0.4	----	----	1	----
June 15–21	22	18	9	1.6	1.6	0.0	2	7	----
June 22–28	33	16	27	1.1	1.8	3.4	2	10	8
June 29–July 5	----	20	20	10.6	5.7	8.6	21	31	28
July 6–12	22	----	22	6.6	4.1	5.2	25	21	23
July 13–19	20	----	17	7.1	1.2	4.2	28	6	18
July 20–26	----	----	0	3.3	2.0	3.1	12	10	13
July 27–Aug. 2	----	----	----	1.9	.6	1.0	7	3	4
Aug. 3–9	----	----	----	.3	.3	.7	1	1	3
Aug. 10–16	----	----	----	.3	.3	.3	1	1	1
Aug. 17–23	----	----	----	.0	.3	.7	----	1	3
Aug. 24–30	----	----	----	.0	.0	.0	----	----	----
Aug. 31–Sept. 6	----	----	----	.0	.3	.0	----	1	----
Sept. 7–13	----	----	----	.0	.3	.0	----	1	----
Total number	----	----	----	----	----	----	89	71	78
Average (percent)	22.3	17.8	20.6	----	----	----	----	----	----

TABLE 27.—*Seasonal fishing mortalities based on an 88.6-percent tag-return efficiency*

Stream and year	Uncorrected fishing mortality	Corrected fishing mortality
	Percent	*Percent*
Arnica Creek:		
1950	7.4	8.4
1951	11.1	12.6
1952	6.5	7.3
1953	8.0	9.1
Average	8.3	9.4
Pelican Creek:		
1949	10.4	11.6
1950	10.3	11.6
1951	8.3	9.4
1952	12.2	13.8
1953	13.5	15.2
Average	10.9	12.3
Clear Creek:		
1953	8.4	9.4
Grouse Creek:		
1951	4.3	4.9
1952	1.2	1.5
1953	4.0	4.7
Average	3.2	3.7
Chipmunk Creek:		
1949	2.2	2.4
1950	4.2	4.9
1952	7.5	8.2
1953	4.8	5.4
Average	4.8	5.2
Trap net:		
1952	17.3	19.5
1953	24.7	28.0
Average	21.0	23.8
Cascades:		
1951	22.3	25.1
1952	17.8	20.0
1953	20.6	23.2
Average	20.2	22.7

Two methods of computing spawning mortality were available for Arnica Creek in 1953 (table 11). The actual count (method 1) was used rather than the estimate (method 2), in this instance. A measure of the reliability of estimate was provided, however. Two methods were used at Pelican Creek in 1952, and the results represent an average mortality figure derived from the two estimates.

The application of data from table 29 provides a measure of the contribution of postspawners from various streams to the sport fishery of the lake (table 28); the surviving number from each spawning run is listed in column 2. When treated with the corrected seasonal fishing mortality figures, the total catch from each stream follows. When the total contribution from each individual stream or the sum for all streams is related to the total lake harvest for the year, the importance of postspawners in the sport fishery is obvious. At no time did the harvest from any individual stream amount to 1 percent of the lake catch. The most sizable contribution by any stream location was 1,297 postspawners from Pelican Creek in 1953, which amounted to only 0.66 percent of the total catch in the lake that year. In 1953 when measurements were made on fish from five streams, their combined totals (2,471 fish) were less than 2 percent of the 195,873 trout estimated to have been taken by fishermen.

TABLE 28.—*Contributions to the lake fishery by the postspawners from several streams, 1949 to 1953*

Stream and tag year	Available to fishery (number)	Corrected fishing mortality (percent)	Contribution to lake fishery	
			Number	Percent
Arnica Creek:				
1950	2, 534	8. 4	213	0. 11
1951	3, 220	12. 6	406	. 19
1952	2, 528	7. 3	185	. 08
1953	2, 462	9. 0	222	. 11
Pelican Creek:				
1949	11, 286	11. 6	1, 309	. 65
1950	5, 910	11. 6	686	. 34
1951	6, 671	9. 3	620	. 30
1952	5, 152	13. 8	711	. 29
1953	8, 648	15. 0	1, 297	. 66
Chipmunk Creek				
1949	10, 457	2. 4	251	. 13
1950	4, 203	4. 9	206	. 10
1952	509	8. 2	42	. 02
1953	4, 075	5. 4	220	. 11
Grouse Creek:				
1951	6, 801	4. 9	333	. 16
1952	2, 856	1. 5	43	. 02
1953	2, 258	4. 7	106	. 05
Clear Creek:				
1953	6, 658	9. 4	626	. 32
All Streams:				
1949	21, 743	2. 4–11. 6	1, 560	. 78
1950	12, 647	4. 9–11. 6	1, 105	. 55
1951	16, 692	4. 9–12. 6	1, 359	. 65
1952	11, 045	1. 5–13. 8	981	. 40
1953	24, 101	4. 7–15. 0	2, 471	1. 26

Stream fishing—Information on stream fishing in tributaries of Yellowstone Lake is available from only one stream, Pelican Creek. Pelican Creek is open to fishing after July 15, as is the case with the other tributaries. This date has been chosen to allow the completion of most spawning activities. Pelican Creek retains a number of postspawners in the stream each summer due to the large size of the watershed, the size of the stream, and the abundance of food. They are subjected to a relatively small amount of stream fishing, as indicated by the number of tags returned from stream anglers. These returns ranged from 0.6 to 1.8 percent of the total number of prespawners tagged. If these returns actually indicate the number of fish that were retained in Pelican Creek, an average of 139 postspawners remained in the stream with 80 in 1953 and 216 in 1949, representing the extremes. The information gained from stream surveys in 1953 and 1954 revealed that these estimates are reasonable.

Spawning runs and opening date of fishery.—An important relation exists between the times of the spawning runs, the location of the tributaries which the spawners ascend, and the opening date of the fishery. An understanding of the sequence of events for the tributary streams of Yellowstone Lake is necessary to good management of the sport fishery.

Adult fish, as they approach the tributary in which they will spawn, become concentrated in limited areas, and become more vulnerable to fishermen if fishing is in progress during the time that the prespawners are assembling. Adequate escapement of prespawners is essential to maintain population numbers. The manipulation of the opening date of the fishery can help to ensure this escapement.

The heaviest fishing pressure at Yellowstone Lake is directed against the northern shoreline areas. Any change in the opening date of the fishery, as was introduced in 1953, would be expected to produce an effect upon the size of the runs into Arnica and Pelican Creeks and the Fishing Bridge area. On the other

TABLE 29.—*Relation of times of spawning runs into trapped streams to the opening of the lake fishery, 1950 to 1953*

[Fishery opened on May 30 through 1952 and on June 15 in 1953]

Stream and year	Spawning runs begin—	Duration of run in days	Fish counted through traps				Total spawning run
			To opening of lake fishery		After opening of lake fishery		
			Number	Percent	Number	Percent	
Arnica Creek:							
1950	May 20	71	854	22	3, 027	78	3, 881
1951	May 8	87	1, 599	34	3, 156	66	4, 755
1952	May 18	73	1, 182	34	2, 341	66	3, 523
1953	May 5	80	2, 882	64	1, 642	36	4, 524
Pelican Creek:							
1950	May 20	68	79	1	14, 997	99	15, 076
1951	May 30	26	905	10	8, 518	90	9, 423
1952	May 30	30	800	12	5, 721	88	6, 521
1953	May 12	58	5, 805	47	6, 638	53	12, 443
Chipmunk Creek:							
1950	June 9	59	0	0	12, 255	100	12, 255
1951	May 30	60	----------	----------	----------	----------	12, 585
1952	May 20	57	6, 660	67	3, 329	33	9, 989
1953	May 31	52	7, 836	61	3, 077	39	7, 836
Grouse Creek:							
1951	June 3	56	0	0	12, 188	100	12, 188
1952	May 26	58	1, 852	27	5, 097	73	6, 949
1953	June 6	46	655	9	6, 896	91	7, 551

hand, trout approaching Chipmunk and Grouse Creeks which are isolated from the intense fishery to the north, should be little affected by this change. Data are available that demonstrate that the 1953 postponement of the opening date of the lake fishery produced changes as anticipated (table 29).

Arnica Creek, in which the run extends from early May to late July, had from 22 to 34 percent of the total count checked through the upstream trap by the onset of fishing in 1950 to 1952. When the opening was changed from May 30 to June 15 in 1953, more than 60 percent of the total run entered the stream before fishing began. Pelican Creek, also a relatively early run stream, but one of shorter duration, experienced an even more striking effect than at Arnica Creek. No more than 12 percent of the total spawning run had been tabulated by May 30 from 1950 through 1952. With the change of regulation in 1953, 47 percent of the fish were counted by June 15. Data from Grouse and Chipmunk Creeks show a smaller change when the opening date of fishing was advanced because the runs occur at a late date. The isolation of the South Arm from heavy boat or shoreline fishing permits only an insignificant harvest of prespawners moving toward Grouse and Chipmunk Creeks. This relation would apply to all streams in the southern area.

The manipulation of opening dates of the fishery in order to guarantee adequate escapement of spawners into streams can be an important management technique when all pertinent factors are known. The proportion of the early season catch that is composed of fish destined to spawn that year, and the determination of the effects on fishing on immature trout in relation to the effects of fishing on prespawners, should be known before precise predictions can be made on the effects of manipulating opening dates.

Natural mortality of postspawners in the lake

Natural mortality in the lake (*lm*) and survival (S') may now be estimated, since methods for determining stream and fishing mortality have been considered. After the stream sojourn, some tagged fish are taken by the fishermen, and those remaining in the lake are unaccounted for until the following year. At that time, some appear in the spawning runs as consecutive spawners or enter the fishery as second-year tag returns. Because so very few spawn a second time, the second-year tag returns provide an estimate of the abundance of surviving postspawners.

First, assume that tag loss during the year after tagging is negligible, as suggested by observations at Arnica Creek. Second, assume that a similar time and space relation exists between the return of first- and second-year tags. This has been shown to be true for all tagging locations except Grouse and Chipmunk Creeks. Then, the fishing mortality estimate for the group of first-year tagged fish is directly applicable to the return of second-year tags. The number of spawners available as second-year tagged fish may be computed in the following fashion, where (1) denotes tags-of-the-year and (2) second-year tags:

$$\frac{Tf_1}{N(Td_1)}=\frac{Tf_2}{NT_2}$$

solving for NT_2,

$$NT_2=\frac{Tf_2\,N(Td_1)}{Tf_1}$$

Second-year tagged fish from Grouse and Chipmunk Creeks were more available to the fishery than first-year tagged fish. This was the only instance where second-year postspawners were subjected to greater fishing pressure than first-year fish. Consequently, groups tagged the year before should incur a higher fishing mortality for the season than fish tagged the same season. Spatial characteristics of their second-year tag returns closely resemble those of the first-year Arnica Creek recoveries; both groups were taken commonly in the West Thumb area. Yet, the typical Chipmunk-Grouse pattern of being caught in the South Arm is retained; thus, the availability of these second-year postspawners is determined by using an arbitrary fishing mortality. The value was set at a mean figure between the estimates for the first-year tag returns of Arnica Creek (upper limit) and Chipmunk and Grouse Creeks (lower limit).

Mortality by postspawners in the lake which may be attributable to natural causes for a period of one year (initial availability to the fishery through the following fishing season), and survival may be computed as follows:

$N(Td)-(Tf)=$ Tags unaccounted for at end of first fishing season (Ts),

and

$$(lm)=(Ts)-NT_2;$$

thus,

$$S'=(Ts)-(lm)$$

A summary of mortality and survival data for all years and all tagging locations is provided in table 30. The information is averaged for each year and for each location. Under normal conditions, a typical spawning run can expect a stream mortality of 48.1 percent. Of those surviving from streams, 8.2 percent will be caught by fishermen. More than half (63.1 percent) of those unaccounted for after fishing is completed will fail to survive through the next summer, with the overwinter period being the critical period. Very few fish spawn a second time. The role that mortality and alternate-consecutive spawning play during and after temporary stream residence may be demonstrated by application of these average mortalities to a spawning run of 1,000 fish (table 18). It is shown that under normal circumstances only 10 fish will function as consecutive spawners, while the potential alternate spawners number 42. By these calculations, the number of fish spawning a second time would not exceed 5.2 percent of the original number.

Noteworthy are the fluctuations in mortality estimates from the average for all areas. Estimates of spawning mortality ranged from 94.9 percent (Chipmunk Creek, 1952) to 21.0 percent (Pelican Creek, 1952). Fishing mortality varied considerably less. Maximum fluctuations from the mean of 8.2 percent for stream locations did not exceed 7 percent.

Table 30.—*Summary of mortalities and survivals expressed as percentage values*

[Figures in parentheses are stream averages for 1951–53]

Stream	Tag population					
	1949	1950	1951	1952	1953	Average
Arnica Creek:						
Stream:						
Spawning mortality	------	38.0	41.2	35.3	50.3	41.2
Survival	------	62.0	58.8	64.7	49.7	58.8
Lake:						
Fishing mortality	------	8.4	12.6	7.3	9.0	9.3
Natural mortality	------	66.6	61.1	87.4	67.2	70.5
Survival	------	25.0	26.3	5.3	23.8	20.2
Pelican Creek:						
Stream:						
Spawning mortality	65.2	60.8	29.2	21.0	30.5	41.3
Survival	34.8	39.2	70.8	79.0	69.5	58.7
Lake:						
Fishing mortality	11.8	11.8	9.3	13.8	15.0	12.2
Natural mortality	35.1	67.8	64.9	72.0	58.6	59.7
Survival	53.3	20.6	25.8	14.2	26.4	28.2
Chipmunk Creek:						
Stream:						
Spawning mortality	23.0	65.7	------	94.9	48.0	57.9
Survival	77.0	34.3	------	5.1	52.0	42.1
Lake:						
Fishing mortality	2.4	4.9	------	8.2	5.4	5.2
Natural mortality	34.2	45.4	------	74.7	58.9	53.3
Survival	63.4	49.7	------	17.1	35.7	41.5
Grouse Creek:						
Stream:						
Spawning mortality	------	------	44.2	58.9	70.1	57.7
Survival	------	------	55.8	41.1	29.9	42.3
Lake:						
Fishing mortality	------	------	4.9	1.5	4.7	3.7
Natural mortality	------	------	59.2	88.1	86.4	77.9
Survival	------	------	35.8	10.4	9.0	18.4
Clear Creek						
Stream:						
Spawning mortality	------	------	------	------	41.2	41.2
Survival	------	------	------	------	58.8	58.8
Lake:						
Fishing mortality	------	------	------	------	9.4	9.4
Natural mortality	------	------	------	------	46.2	46.2
Survival	------	------	------	------	44.4	44.4
Trap net:						
Lake:						
Fishing mortality	------	------	------	19.5	28.0	23.8
Natural mortality	------	------	------	63.2	53.8	58.5
Survival	------	------	------	17.3	18.2	17.8
Yellowstone River Cascades:						
River:						
Fishing mortality	------	------	25.1	20.0	23.2	22.8
Natural mortality	------	------	43.6	51.0	65.2	53.3
Survival	------	------	31.3	29.0	11.6	24.0
Average for year:						
Stream						
Spawning mortality	44.1	54.8	38.2	52.5	48.0	48.1
Survival	55.9	45.2	61.8	47.5	52.0	51.9
Lake and River combined:						
Fishing mortality	7.0 ------	8.3 ------	13.0 (8.9)	11.7 (7.7)	13.5 (8.7)	11.6 (8.2)
Natural mortality	34.7 ------	59.8 ------	57.2 (61.7)	72.7 (80.6)	62.3 (63.4)	61.4 (63.1)
Survival	58.4 ------	31.8 ------	29.8 (29.3)	15.6 (11.8)	24.2 (27.9)	27.0 (28.6)

Estimates of fishing mortality for the trap net and Cascades tagging ranged from 19.5 percent to 28.0 percent, and the mean was 23.2 percent. The maximum and minimum estimates for natural mortality in the lake were 88.1 percent and 34.2 percent. This fluctuation did not exceed 28 percent of the mean estimate of 61.4 percent.

The principal factors influencing mortality in lakes are similar to those discussed earlier for streams. Other factors include age, sex, general physical condition, and the degree of parasitism for each fish. The sex of cutthroat trout was shown to be related to the relative amount of weight lost and the length of time spent in the stream. By losing a small amount of body weight exclusive of sex products and by spending a small amount of time in the stream, the adult female should emerge from spawning in better physical condition than the male. This situation was probably the cause of the differential sex mortality at Arnica Creek in 1953. Theoretically, then, the female would be in better condition for overwinter survival. Adults surviving spawning in best condition, or those having the longest and most productive feeding period after returning to the lake may not only experience a higher survival rate, but also represent the best source of consecutive spawners. With regard to age and mortality, 4-year-old fish spawning for the first time would spend a year longer in the lake as feeders than 3-year-old initial spawners. Consequently, they might be in better physical condition before and after spawning. These factors may interact in a complex fashion, providing a basis for differential survival rates, alternate-consecutive spawning ratios, and a relatively high rate of natural lake mortality.

Dispersal of postspawners.—Apparent migration of adult fish from the various tagging locations was indicated by the distribution of tags returned from the sport fishery. For purposes of analysis, five areas were delimited in the Methods section either by geographic or fishery characteristics, or both (fig. 2).

In terms of fishing pressure, the Fishing Bridge area represents the focal point of the most concentrated fishing effort on the lake (Cope, 1957). If fishing here may be considered "very heavy," then by comparison fishing effort in the northern area could be termed "heavy." The fishery in West Thumb might be classified as "moderate," while the southern area supports "light" fishing pressure. Angling on the Yellowstone River most closely approximates that of the northern area and therefore may be called "heavy."

Tagged fish taken by fishermen were tabulated into first- and second-year returns by stream group and for individual years, and apportioned into areas where captured (appendix table 12). In some cases, the total return of tags for the year (table 21) may be greater than that used in the analysis of tag return areas, because tags are sometimes returned from the fishery without information concerning the date and place of capture. For this reason, questionable tags were eliminated from consideration at this time. Therefore, this presentation depicts the average relative contribution of tagged fish from various streams to designated areas.

Tag return areas for postspawners.—Arnica Creek fish were taken mostly in the West Thumb area. The lake section adjacent to the mouth of Arnica Creek accounted for the greatest number of first- and second-year tagged fish. Pumice Point and the dock area in West Thumb proper, Rock Point and Bridge Bay in the northern half, and the southern part of the Fishing Bridge area were other focal points for the return of Arnica Creek tags.

Second-year tags entered the western shoreline of the northern area to a greater extent than did first-year tags. For purposes of computing lake mortality and survival, however, the movement could not account for second-year tagged fish being exposed to a markedly different fishing pressure.

The northern area accounted for an average of about 65 percent of first- and second-year tags from Pelican Creek. The greatest number from any one location came from the heavily fished southern portion of the Fishing Bridge area. Only an occasional Pelican Creek fish was detected in the Yellowstone River proper. The most productive localities for the return of Pelican Creek tags were Steamboat Point, Bridge Bay, and Mary Bay, all on the north shore of Yellowstone Lake. A slight spatial dispersion of second-year tags was detected, but not to the extent of introducing these postspawners to a radically different fishing pressure.

Results of tagging at Clear Creek suggest a generalized migration into the northern area. Rock Point, Bridge Bay, Stevenson Island, the southern part of the Fishing Bridge area, and the shoreline adjacent to Clear Creek itself were important localities for tag returns. More marked fish from this stream were caught in Bridge Bay than in any other locality. No indication of a movement of Clear Creek fish into the Yellowstone River was detected.

Tag recoveries of Grouse and Chipmunk Creek fish demonstrated the southern half and West Thumb areas to be the most important parts of the lake with regard to capture and apparent migration. Large boats in the South Arm landed the majority of tagged fish from these two tributaries. Occasionally marked fish were were taken in the Southeast Arm, at Bridge Bay, Dot Island, Wolf Point, and in the vicinity of the West Thumb dock. Because second-year tags dispersed into the West Thumb area to a greater extent than did tags of the year, modification in the method of estimation of natural mortality in the lake was necessary.

Considering the unequal distribution of fishing effort on Yellowstone Lake, these data support a view that postspawners from various streams tend to localize in lake areas nearest the parent stream itself, regardless of time. No evidence is at hand to indicate any appreciable movement of "lake" fish into the Yellowstone River below Fishing Bridge. Both first- and second-year tags typically are caught in the same general areas of the lake, with some exception of adult fish from Chipmunk and Grouse Creeks.

Tagged fish from the trap net and Cascades operations have also been considered in relation to locality of capture (table 31). The Fishing Bridge and Yellowstone River areas were the ones primarily involved in the analysis. Because of the small number of returns from the three lake areas, they were combined and designated the Yellowstone Lake area. These data led to a good understanding of the movements of "river" and "lake" fish.

About half of all Cascades tags recovered were taken in the Fishing Bridge area, and were distributed rather uniformly throughout the area. Second-year tagged fish appeared in the lake fishery in larger relative numbers than did first-year tags. An average of 10.2 percent of the returns from first-year tags were taken in Yellowstone Lake proper, while 27.8 percent of the second-year tags were caught in the lake. An average of 7.1 percent of the trap net fish recovered the first year were caught in the river; as second-year tags, one-fourth of those captured came from the river. More trap net fish were taken in the Fishing Bridge area than in all other areas combined. This operation furnished additional proof that relatively little movement of lake fish into the river system took place. Second-year tags dispersed more than did first-year tags.

Time intervals between tagging and capture.—Apparently the movement of trap-net fish back into the lake from the Fishing Bridge area made them much less available to capture thereafter than were the Cascades fish moving downriver. That is, "river" fish, having moved back into the river after spawning near Fishing Bridge, were still confined to a relatively limited area and largely exposed to the full extent of the fishing pressure developed there. Trap-net fish, on the other hand, retreated into an extensive lake area that was fished mostly along certain portions of the shoreline. A comparison of the fishing success from both groups provides additional evidence that the migration characteristics probably determined postspawner availability.

The effect of postspawner dispersion from Arnica Creek, the trap net, and the Cascades is further demonstrated by measuring the time between availability of tagged fish to the fishery and their capture (table 32). The usable tag returns from Arnica Creek from 1950 to 1952 were combined in order to obtain a sufficient number of returns for this purpose. The length of time from the moment of availability to the fishery to date of capture for each fish was arranged into weekly intervals. The initial moment of availability began with the opening date of the lake fishery if the

TABLE 31.—*Fishery returns by areas from Cascades and trap net tagging*

Location and year of tagging	Percentage of total returns from—						Total number of returns	
	Yellowstone Lake area		Fishing Bridge area		Yellowstone River area			
	1st-year	2d-year	1st-year	2d-year	1st-year	2d-year	1st-year	2d-year
Cascades:								
1951	10. 5	38. 1	38. 4	42. 9	51. 2	19. 0	86	21
1952	14. 9	20. 8	52. 2	54. 2	32. 8	25. 0	67	24
1953	5. 6	22. 2	61. 1	33. 3	33. 3	44. 5	72	9
Three years	10. 2	27. 8	49. 8	46. 3	40. 0	25. 9	225	54
Trap net:								
1952	23. 7	10. 0	69. 5	60. 0	6. 8	30. 0	59	20
1953	10. 4	18. 2	82. 1	63. 6	7. 5	18. 2	67	11
Two years	16. 7	12. 9	76. 2	61. 3	7. 1	25. 8	126	31

TABLE 32.—*Summary of time intervals before capture expressed in weekly increments from the moment of availability to the fishery*

Time of capture by weekly periods	Percent of total tagged in—									
	Arnica Creek			Cascades				Trap net		
	1950–52	1953	All years	1951	1952	1953	All years	1952	1953	All years
May 4–10	6.7	17.4	13.1	49.4	13.0	43.8	35.6	52.3	47.1	49.6
May 11–17	17.8	11.6	14.0	15.9	15.9	21.9	18.0	25.4	33.8	29.8
May 18–24	15.5	16.0	15.8	18.9.	14.5	15.1	16.1	15.9	11.8	13.7
May 25–31	11.1	5.8	7.9	11.6	26.1	5.6	14.2	4.8	4.4	4.6
June 1–7	6.7	4.3	5.3	1.4	11.6	5.6	6.2	------	2.9	1.5
June 8–14	11.1	16.0	14.0	1.4	5.8	4.1	3.8	1.6	------	.8
June 15–21	11.1	13.0	12.3	1.4	5.8	1.3	2.8	------	------	------
June 22–28	11.1	11.6	11.4	------	4.4	1.3	1.9	------	------	------
June 29–July 5	6.7	4.3	5.3	------	2.9	1.3	1.4	------	------	------
July 6–12	2.2	------	.9	------	------	------	------	------	------	------
Total number of tags	45	69	114	69	69	73	211	63	68	131

fish had been liberated into the lake or river prior to that date. If liberation into the lake took place after fishing had begun, then the date of liberation became the moment of availability.

About half of the recovered trap-net fish were caught within the first week following availability. As a rule, less than 7 percent were caught after the third week of exposure to the fishery. This pattern also generally applied to fish from the Cascades, where most of the marked fish were recovered within a week or two after becoming available. However, Cascades fish were caught over a longer period of time than were the trap-net fish for reasons previously suggested. This condition emphasizes the role of dispersion as it determines the rate of tag return. For instance, an average of about 30 percent of the Cascades fish landed from 1951 to 1953 were taken after the third week of availability, while only 6.9 percent of the trap-net fish normally entered the creel after 3 weeks' exposure to the fishermen. The deviation of the results of Cascades tagging in 1952 from the 1951 and 1953 data probably resulted from the effects of the earlier spawning migration in 1952. An earlier movement of mature fish may have allowed a greater number, possibly the bulk of the run, to appear in the Fishing Bridge area and disperse in the river before the period of most intense fishing. An extension of the length of time between availability and capture would result, as indicated by the data.

The evidence at hand shows that the fishery in and around the Fishing Bridge area is dependent on the influx of mature fish from the river and lake. The opening date of the river fishery, July 1, occurs during the period of spawning migration into this area. The movement up the river seems to precede that from the lake by a week or two. On July 1, heavy fishing pressure is imposed upon the limited area where the accumulation of fish has been taking place. Measurements of fishing intensity and the creel census estimates have demonstrated the great success of the Fishing Bridge area anglers during the first few weeks of July (Moore, Cope, and Beckwith, 1952). The angling success appears to be related to the movement of fish into the area (table 33). The data suggest that in 1952 early spawning runs by "river" and "lake" fish allowed a considerable escapement from the area by postspawners

TABLE 33.—*Relation between the Fishing Bridge catch and the trap-net and Cascades fishing-intensity estimates*

Year	Total catch (fish)	Peak rate of fishing (percent)		Fishing mortality (percent)	
	Fishing Bridge	Trap net	Cascades	Trap net	Cascades
1950	6,417	------	------	------	------
1951	8,938	------	10.6	------	22.3
1952	4,865	7.9	5.7	17.5	17.8
1953	5,388	14.1	8.6	24.7	20.6

back into their respective areas of origin before fishing began. This condition is generally reflected in the Fishing Bridge catch and the fishing intensity measurements, although other factors interact here to obscure the association between fishing mortality rates and Fishing Bridge catches.

The estimates of natural lake mortality for Cascades and trap-net fish, including the effects of spawning, compare favorably with the natural mortality (*lm*) estimates from streams. The sources of spawning mortality which operate in streams are much less emphasized around the river outlet. Furthermore, many adults that otherwise would have succumbed to spawning are prematurely taken by the Fishing Bridge area fishermen.

Second-year river and lake tags appeared in the Fishing Bridge area fishery in greater relative proportion than did the first-year tags. This appears to refute the alternate-consecutive spawning concept and the theory that the Fishing Bridge fishery is almost completely dependent on the movements of initial spawners. Dispersion of second-year tagged fish has been demonstrated. This may be due to a migration of postspawners toward feeding areas in the lake near the outlet. Moreover, a higher percentage of consecutive spawners may dominate these two groups, in which a greater movement to the Fishing Bridge area as second-year tags would result. A more uniform and favorable aquatic environment in the river could result in postspawners being in better physical condition and lead to a greater tendency for consecutive spawning.

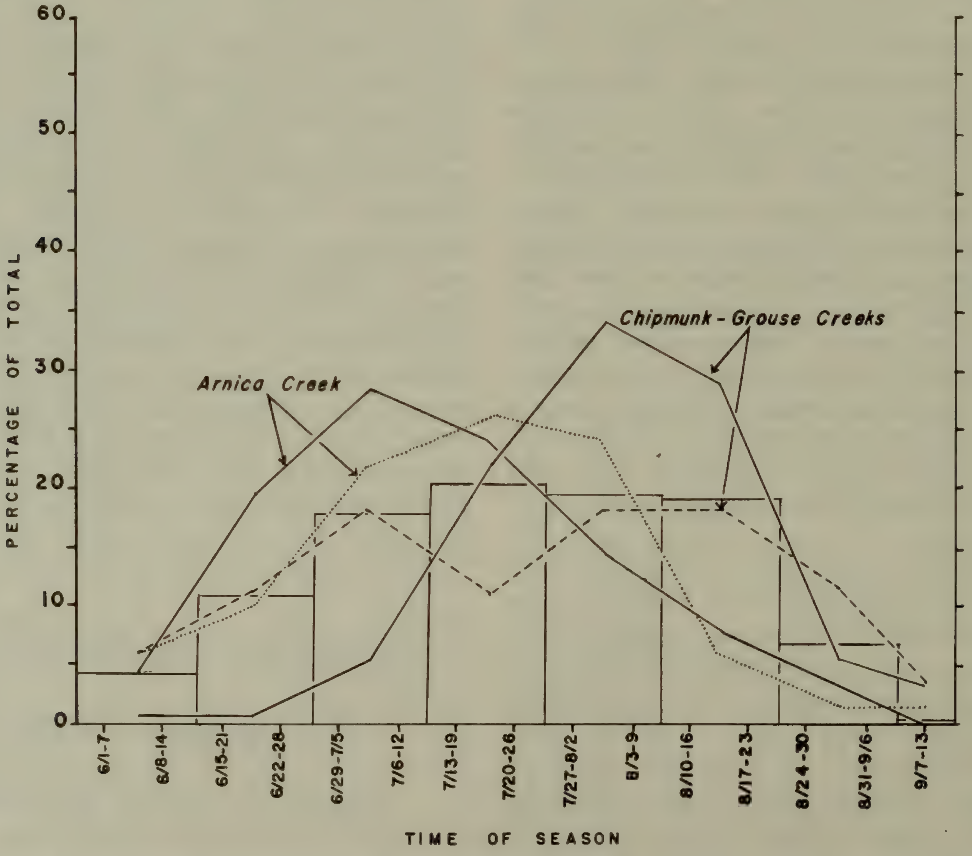

FIGURE 6.—Arnica and Grouse and Chipmunk Creeks average annual returns, compared with the West Thumb area fishery catch, 1950 to 1953. Histogram represents average annual catch, solid lines are first-year returns, and broken lines are second-year returns.

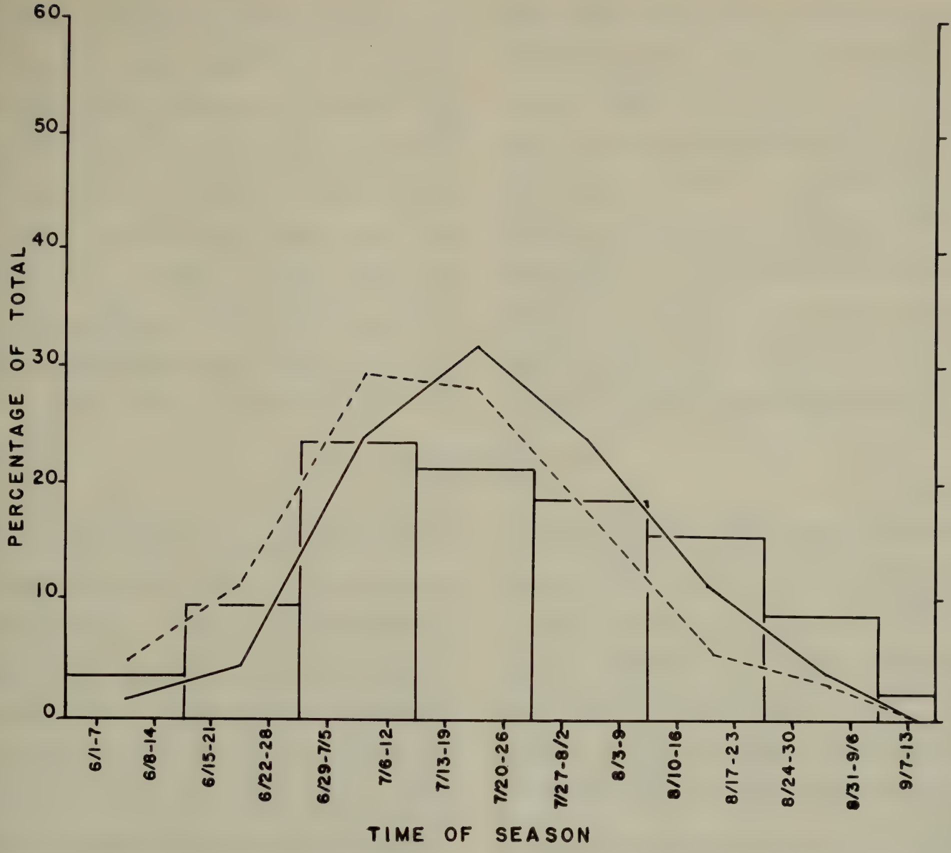

Figure 7.—Pelican Creek average annual returns of tagged fish, compared with the distribution of the Fishing Bridge area fishery catch, 1949 to 1953. Histogram represents average annual catch, solid lines are first-year returns, and broken lines are second-year returns.

West Thumb fishery.—Tagged fish from Arnica and Chipmunk-Grouse Creeks were characteristically taken in the West Thumb area. First year postspawners from the South Arm usually entered the fishery late in the season. As second-year tags, they were more uniformly harvested throughout the season. Postspawners from Arnica Creek followed a similar pattern both as first- and as second-year tags. Period returns showed them to be taken much earlier in the season than were Chipmunk and Grouse Creek fish (fig.6, appendix table 7).

The West Thumb fishery is unusual in that a successful catch is extended over a longer period of time than any of the other components of the Yellowstone fishery (table 5). The tag returns from Arnica Creek and Chipmunk and Grouse Creeks help explain this situation. The endemic population resulting from reproduction within the boundaries of West Thumb is a small one. The presence of so few spawning tributaries in that area, Arnica Creek being one of the two largest, substantiates this conclusion. Therefore, the highly successful early-season fishery of the northern portion of the lake and in the Yellowstone River, which appears to be largely dependent on prespawning movement of adult fish, is lacking in West Thumb (fig. 7). The fishery of the Fishing Bridge area takes postspawners from Pelican Creek fairly early in the season, compared with the entry of Chipmunk and Grouse Creek postspawners into the West Thumb fishery. The West Thumb fishery must be rather dependent on a recruitment or migration of fish from other areas later in the season. This is suggested by the period returns of Grouse-Chipmunk and Arnica Creeks when compared with the creel census from the West Thumb area, where most of the tags are returned. The early fishery is supported by adult fish before and after spawning. The dispersion of postspawners later in the season, as indicated by the Arnica Creek period returns, would normally lead to a decrease in catch at that time, as was demonstrated elsewhere. However, postspawners from Grouse and Chipmunk Creeks are shown to re-enter the lake during the latter part of July, and appear in the West Thumb fishery several weeks later. Hence, the late season movement of fish into West Thumb probably stems from postspawners immature fish, and recruitment of small adults. Not only do they move in from the South Arm and Southeast Arm, but also from other portions of the southern section of Yellowstone Lake. Nevertheless, this recruitment, as suggested by tag returns, at best extends a limited fishery into one of moderate proportions.

SUMMARY

The Fish and Wildlife Service studied factors associated with mortality of eggs, immature fish, spawners, and postspawners of the Yellowstone cutthroat trout in Yellowstone Lake (Wyoming) and its tributaries. These efforts were connected with broad studies intended to determine the number of trout that can be removed from these waters without damaging the populations.

Background material is furnished to describe the biology of the cutthroat and its physical and biological environment in the lake and streams. Water temperatures, history of trout management, sizes of the spawning runs, sex ratios in favor of the females in spawning runs, and the characteristics of the extensive and intensive sport fishery are discussed.

Methods of measuring mortality of eggs and fry by examination of redds, mortality of fry, fingerlings, and adults by counting through upstream-downstream traps, and mortality of adults by tagging with Petersen disks and recovery at traps and in the fishery, are described. Five methods of computation are given to show ways of determining mortality of adults in streams by trap counts and by recovery of tags from the fishery.

Deaths of cutthroat eggs in the gravel in Arnica Creek ranged from 60 to 75 percent to the time of hatching, 36 to 40 days after deposition. Deaths to the time the swimming fry and fingerlings left Arnica Creek and entered Yellowstone Lake averaged 99.6 percent of the original eggs deposited in the stream. Thus, for every female spawner with an average of 1,107 eggs, 4.54 immature trout were alive in September of the first year. Of these, 1.67 must survive to become spawners in order to maintain the population in numerical stability over a period of time.

Efficiency of return of tags recovered from fishermen was determined to be 88.6 percent. Tagged adults in Arnica Creek were found to have a maximum of 12 percent higher death rate in the stream than did fish not tagged. Male and female trout were observed to suffer essentially the same mortalities. The average mortality of spawners in 5 streams during 5 years was 48.1 percent. The upstream-downstream tagging method, devised for these studies for measurement of upstream mortality, was tested against upstream-downstream counts in Arnica Creek and found to give accurate estimates. No explanation is found to explain the uneven sex ratio in the spawning runs (1.47 females to 1 male) or in the fishery (1.62 females to 1 male). Males stayed upstream in Arnica Creek for an average of 17.3 days; females averaged 7.3 days. Evidently, many male spawners service more than one female. Males lose an average of 18.5 percent of their body

weight while on the spawning run; females lose 21.3 percent. The peaks of spawning runs occurred when water temperatures reached the vicinity of 55° F. The early portion of the spawning run had relatively high mortality, the peak of the run suffered fewer deaths, and the last part, during periods of increasing water temperatures, sustained heavy losses. Spawners spent less time in the stream as the season progressed. Fish that were artificially spawned and returned to upstream water had lower mortality rates than fish that spawned naturally in Chipmunk Creek, but had higher rates in Pelican Creek. Of trout that returned to spawn a second time, an average of 6.9 percent of those alive spawned consecutively (the next year), while 22.8 percent of those alive spawned alternately (2 years later). Relatively few fish spawned a second time; 1.22 percent of the runs were fish that had spawned before.

Of the fish in Arnica Creek spawning runs, 48.6 percent died in the stream, 40.2 percent died later in the lake of natural causes, 7.6 percent were caught by fishermen, and 3.6 percent were alive 2 years later.

The white pelicans that breed on the Molly Islands in the Southeast Arm of Yellowstone Lake consume from 300,000 to 450,000 pounds of fish each year. Most of these fish are cutthroat trout from Yellowstone Lake. The fish are caught principally in the south end of the lake, near the mouths of streams and on the spawning beds. Other predators take lesser tolls, the osprey and the bear being two most serious rivals to the pelican.

Postspawners from five streams contributed less than 2 percent of the fishery. Tag returns from postspawners showed that fishing mortality among fish released from a trap net in the lake and on fish tagged at the Cascades on the Yellowstone River was several times greater than mortalities estimated for postspawners from smaller streams. Fish were released directly into areas with heavy fishing at the former locations; fish were able to disperse into the lake from tributary streams, and longer times before capture were characteristic of the stream populations. Average fishing mortality in fish tagged in the lake and river from 1949 to 1953 was 11.6 percent of the fish available to the fishermen.

Tagging showed that many trout move upstream to the Fishing Bridge area to spawn and return downriver after spawning; also, many move from the lake to the Fishing Bridge area to spawn and reenter the lake after spawning. There was little passage of "river" fish into the lake or "lake" fish into the river. Tagging also indicated that many postspawners from Chipmunk and Grouse Creeks move into West Thumb before entering the fishery. This migration pattern suggests a reason for the late season fishery in the West Thumb area. The heaviest harvest of fish takes place in the northern part of the lake.

Changing the opening date of fishing from May 30 to June 15 in 1953 resulted in considerable escapement of spawners into streams.

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APPENDIX TABLES

Appendix table 1.—*Fishing intensity data obtained from upstream tagging at Arnica Creek, 1950–52*

Time period	Tag group checked downstream			Tagged fish checked downstream by periods				Rates of fishing
	Tag group	Fishing returns	Fishing mortality	Time group	Period fishing returns		Cumulative availability	
1950	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Percent*
May 4–10								
May 11–17								
May 18–24	12	4	33					
May 25–31	47	3	6					
June 1–7 [1]	176	10	6					
June 8–14	218	9	4					
June 15–21	41	4	10	312	0	0	312	0.
June 22–28	41	8	20	79	2	5	391	.
June 29–July 5	12	2	17	112	12	27	501	2.
July 6–12	10	2	20	13	7	16	502	1.
July 13–19	12	2	17	8	6	14	503	1.
July 20–26	29	0	0	4	5	11	501	1.
July 27–Aug. 2				29	3	7	525	.
Aug. 3–9				8	3	7	530	.
Aug. 10–16				25	3	7	552	.
Aug. 17–23				4	1	2	553	.
Aug. 24–30				4	1	2	556	.
Aug. 31–Sept. 6					1	2	555	.
Sept. 7–13							554	.
Total	598	44	7.4	598	44	7.4	554	
1951								
May 4–10	3	0	0					
May 11–17	53	12	23					
May 18–24	79	10	13					
May 25–31	206	27	13	3				
June 1–7	135	8	6	135			138	.
June 8–14	71	7	10	243	5	6	381	1.3
June 15–21	34	5	15	135	8	10	511	1.6
June 22–28	45	7	16	70	12	15	573	2.1
June 29–July 5	37	3	8	31	10	12	592	1.7
July 6–12	28	2	7	37	7	9	619	1.1
July 13–19	20	1	5	42	12	15	654	1.8
July 20–26	11	0	0	11	11	13	653	1.7
July 27–Aug. 2	8	0	0	20	2	2	662	.3
Aug. 3–9	6	0	0	6	6	7	666	.9
Aug. 10–16				3	4	5	663	.6
Aug. 17–23					2	2	659	.3
Aug. 24–30					2	2	657	.3
Aug. 31–Sept. 6					1	1	655	.2
Sept. 7–13							654	.0
Total	736	82	11.1	736	82	11.1	654	
1952								
May 5–10								
May 11–17								
May 18–24	62	7	11	29				
May 25–31	131	13	10	78				
June 1–7	210	12	6	99	0		206	.0
June 8–14	125	5	4	67	5	13	273	1.8
June 15–21	47	1	2	110	6	15	378	1.6
June 22–28	13	0	0	142	5	13	514	1.0
June 29–July 5	8	0	0	21	7	18	530	1.3
July 6–12	9	1	11	40	8	20	563	1.4
July 13–19	6	1	17	5	2	5	560	.4
July 20–26	5	0	0	11	2	5	569	.4
July 27–Aug. 2	4	0	0	13	1	2	580	.2
Aug. 3–9				5	0		584	.0
Aug. 10–16				0	3	8	584	.5
Aug. 17–23				0	1	2	581	.2
Aug. 24–30				0			580	.0
Aug. 31–Sept. 6							580	.0
Sept. 7–13							580	.0
Total	620	40	6.5	620	40	6.5	620	

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[1] Fishing began on May 30.

Appendix table 2.—*Fishing intensity data obtained from tagging at Arnica Creek, 1953*

SECTION 1—UPSTREAM TAGGING

Time period	Tag group checked downstream			Tagged fish checked downstream by period—time group					Fishing returns (number)	Fishing mortality (percent)
	Tag group (number)	Fishing returns (number)	Fishing mortality (percent)	Time group (number)	Period fishing returns Number	Period fishing returns Percent	Cumulative availability	Rates of fishing (percent)		
May 4–10	13	2	15	-----	-----	-----	-----	-----	-----	-----
May 11–17	37	4	11	7	-----	-----	-----	-----	1	14
May 18–24	35	2	6	5	-----	-----	-----	-----	0	0
May 25–31	98	11	11	29	-----	-----	-----	-----	5	17
June 1–7	105	11	10	49	-----	-----	-----	-----	6	12
June 8–14	77	3	4	96	-----	-----	-----	-----	11	11
June 15–21	51	6	12	104	4	10	290	1.4	10	10
June 22–28[1]	35	1	3	30	2	5	316	.6	1	3
June 29–July 5	33	0	0	60	9	23	374	2.4	5	8
July 6–12	13	0	0	105	4	10	470	.9	1	1
July 13–19	7	0	0	13	4	10	479	.8	0	0
July 20–26	1	0	0	5	5	13	480	1.0	0	0
July 27–Aug. 2	-----	-----	-----	2	5	13	477	1.0	0	0
Aug. 3–9	-----	-----	-----	-----	4	10	472	.8	-----	-----
Aug. 10–16	1	0	0	-----	2	5	468	.4	-----	-----
Aug. 17–23	-----	-----	-----	1	1	3	467	.2	0	0
Aug. 24–30	-----	-----	-----	-----	-----	-----	466	.0	-----	-----
Aug. 31–Sept. 6	-----	-----	-----	-----	-----	-----	466	.0	-----	-----
Sept. 7–13	-----	-----	-----	-----	-----	-----	466	.0	-----	-----
Total	506	40	7.9	506	40	-----	466	-----	40	7.9

[1] Fishing began on June 15.

SECTION 2—DOWNSTREAM TAGGING

Time period	Tagged fish checked downstream by period—time group					Time group fishing returns (Number)	Time group fishing mortality (Percent)
	Time group (Number)	Period fishing returns Number	Period fishing returns Percent	Cumulative availability (Number)	Rates of fishing (Percent)		
May 4–10	-----	-----	-----	-----	-----	-----	-----
May 11–17	-----	-----	-----	-----	-----	-----	-----
May 18–24	7	-----	-----	-----	-----	0	0
May 25–31	14	-----	-----	-----	-----	2	14
June 1–7	25	-----	-----	-----	-----	3	12
June 8–14	60	-----	-----	-----	-----	5	8
June 15–21	69	5	17	175	2.9	12	17
June 22–28[1]	23	2	7	193	1.0	2	9
June 29–July 5	41	-----	-----	232	.0	4	10
July 6–12	97	3	10	329	.9	1	1
July 13–19	9	4	14	335	1.2	0	0
July 20–26	2	4	14	333	1.2	0	0
July 27–Aug. 2	3	6	20	332	1.8	0	0
Aug. 3–9	1	4	14	327	1.2	0	0
Aug. 10–16	-----	-----	-----	323	.0	-----	-----
Aug. 17–23	-----	1	3	323	.3	-----	-----
Aug. 24–30	1	-----	-----	323	.0	0	0
Aug. 31–Sept. 6	1	0	-----	324	.0	0	0
Sept. 7–13	-----	-----	-----	324	.0	-----	-----
Total	353	29	-----	324	-----	29	8.2

[1] Fishing began on June 15.

APPENDIX TABLE 2.—*Fishing intensity data obtained from tagging at Arnica Creek, 1953*—Continued

SECTION 3—UPSTREAM AND DOWNSTREAM TAGGING

[Combined comparable portions of Sections 1 and 2.]

Time period	Tagged fish checked downstream by period—time group					Time group fishing returns	Time group fishing mortality
	Time group (Number)	Period fishing returns		Cumulative availability (Number)	Rates of fishing (Percent)	(Number)	(Percent)
		Number	Percent				
May 4–10	---	---	---	---	---	---	---
May 11–17	7	---	---	---	---	1	14
May 18–24	12	---	---	---	---	0	0
May 25–31	43	---	---	---	---	7	16
June 1–7	74	---	---	---	---	9	12
June 8–14	156	---	---	---	---	16	10
June 15–21	173	9	13	465	1.9	22	13
June 22–28 [1]	53	4	6	509	.8	3	6
June 29–July 5	101	9	13	606	1.5	9	9
July 6–12	202	7	10	799	.9	2	1
July 13–19	22	8	12	814	1.0	0	0
July 20–26	7	9	13	813	1.1	0	0
July 27–Aug. 2	5	11	16	809	1.4	0	0
Aug. 3–9	1	8	12	799	1.0	0	0
Aug. 10–16	---	2	3	791	.3	---	---
Aug. 17–23	1	2	3	790	.3	0	0
Aug. 24–30	1	0	---	789	.0	0	0
Aug. 31–Sept. 6	1	0	---	790	.0	0	0
Sept. 7–13	0	0	---	790	.0	---	---
Total	859	69	---	790	---	69	8.0

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[1] Fishing began on June 15.

APPENDIX TABLE 3.—*Rates of fishing obtained from downstream tagging at Pelican Creek, 1952*

Time period	Number tagged	Period returns		Cumulative availability (Number)	Rate of fishing (Percent)	Time group fishing mortality	
		Number	Percent			Number	Percent
June 1–7	---	---	---	---	---	---	---
June 8–14	---	---	---	---	---	---	---
June 15–21	64	---	---	64	0.0	7	11
June 22–28	---	---	---	64	0.0	---	---
June 29–July 5	103	1	5	167	0.6	13	13
July 6–12	0	5	25	166	3.0	---	---
July 13–19	0	3	15	161	1.9	---	---
July 20–26	0	5	25	158	3.2	---	---
July 27–Aug. 2	0	2	10	153	1.3	---	---
Aug. 3–9	0	1	5	151	0.7	---	---
Aug. 10–16	0	3	15	150	2.0	---	---
Aug. 17–23	0	---	---	147	0.0	---	---
Aug. 24–30	0	---	---	147	0.0	---	---
Aug. 31–Sept. 6	0	---	---	147	0.0	---	---
Sept. 7–13	0	---	---	147	0.0	---	---
Total	167	20	100	147	---	20	12.0

Appendix table 4.—*Rates of fishing obtained from downstream tagging at Chipmunk and Grouse Creeks, 1952 and 1953*

Time period	1952				1953			
	Number tagged (Number)	Period returns (Number)	Cumulative availability (Number)	Rate of fishing (Percent)	Time group (Number)	Period returns (Number)	Cumulative availability (Number)	Rate of fishing (Percent)
June 1- 7	(1)							
June 8-14					(1)			
June 15-21	17		17	0.0				
June 22-28	32		49	0.0				
June 29-July 5			49	0.0	64		64	0.0
July 6-12	29	1	78	1.3	30		94	0.0
July 13-19	94		171	0.0	22		116	0.0
July 20-26	68	4	239	1.7	69		185	0.0
July 27-Aug. 2	87	2	322	0.6	99	2	284	0.7
Aug. 3- 9		3	320	0.9		5	282	1.8
Aug. 10-16		1	317	0.3		2	277	0.7
Aug. 17-23			316	0.0		2	275	0.7
Aug. 24-30			316	0.0			273	0.0
Aug. 31-Sept. 6			316	0.0		1	273	0.4
Sept. 7-13			316	0.0			272	0.0
Total	327	11	316		284	12	272	

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[1] Opening of fishing season.

Appendix table 5.—*Period fishing returns obtained from tagging at Pelican Creek, 1949 to 1953*

[Percentage of total returns for each season given in parentheses]

Time period	Number of fish returned from—											
	1949 tagging		1950 tagging		1951 tagging		1952 tagging		1953 tagging		Total tagging	
	1949	1950	1950	1951	1951	1952	1952	1953	1953	1954	First year	Second year
June 1-7		2(14)	1(2)			1(4)					1(0.3)	3(3)
June 8-14	4(15)		1(2)			2(7)	1(1)				6(2)	2(2)
June 15-21			1(2)			2(7)	4(4)	2(14)	1(1)	3(10)	6(2)	7(7)
June 22-28	2(7)		4(7)	1(11)	2(3)		3(3)			4(13)	11(3)	5(5)
June 29-July 5	7(26)	2(14)	4(7)	3(33)	2(3)	5(18)	15(16)	1(7)	9(8)	3(10)	37(10)	14(15)
July 6-12	4(15)		3(6)	3(33)	9(12)	3(11)	17(18)	4(29)	15(13)	4(13)	48(13)	14(15)
July 13-19	2(7)	3(21)	9(17)		9(12)	2(7)	5(5)	2(14)	13(11)	4(13)	38(10)	11(11)
July 20-26	5(19)	1(7)	13(24)		17(23)	8(29)	14(15)	3(21)	29(25)	3(10)	78(21)	15(16)
July 27-Aug. 2	2(7)	3(21)	8(15)	1(11)	10(13)	2(7)	12(13)	1(7)	20(17)	4(13)	52(14)	11(11)
Aug. 3-9	1(4)		7(13)		7(9)	1(4)	8(9)	1(7)	12(10)	3(10)	35(10)	5(5)
Aug. 10-16		1(7)	2(4)	1(11)	6(8)		7(8)		11(9)	2(6)	26(7)	4(4)
Aug. 17-23			1(2)		6(8)	1(4)	2(2)		4(3)	1(3)	13(4)	2(2)
Aug. 24-30		2(14)			7(9)	1(4)	4(4)		2(2)		13(4)	3(3)
Aug. 31-Sept. 6												
Sept. 7-13												
Total	27	14	54	9	75	28	92	14	116	31	364	96

Appendix table 6.—*Period fishing returns obtained from the combined Chipmunk Creek and Grouse Creek data, 1949 to 1953*

[Percentage of total returns for each season given in parentheses]

Time period	Number of fish returned from—											
	1949 tagging [1]		1950 tagging [1]		1951 tagging [2]		1952 tagging [3]		1953 tagging [3]		Total tagging	
	1949	1950	1950	1951	1951	1952	1952	1953	1953	1954	First year	Second year
June 1-7	--------	--------	--------	1(8)	--------	--------	--------	--------	--------	--------	--------	1(1)
June 8-14	--------	2(6)	--------	1(8)	1(3)	1(7)	--------	--------	--------	--------	1(1)	4(5)
June 15-21	--------	1(3)	--------	1(8)	--------	3(20)	--------	--------	--------	--------	--------	5(6)
June 22-28	--------	2(6)	--------	--------	--------	1(7)	1(6)	--------	--------	1(10)	1(1)	4(5)
June 29-July 5	--------	3(9)	--------	2(17)	--------	1(7)	1(6)	1(20)	1(4)	--------	2(2)	7(9)
July 6-12	1(9)	2(6)	1(5)	1(8)	1(3)	1(7)	1(6)	1(20)	--------	2(20)	4(4)	7(9)
July 13-19	3(27)	1(3)	--------	2(17)	2(7)	1(7)	--------	--------	2(7)	1(10)	7(7)	5(6)
July 20-26	2(18)	1(3)	1(5)	1(8)	5(17)	1(7)	4(25)	1(20)	3(11)	--------	15(15)	4(5)
July 27-Aug. 2	4(36)	3(9)	3(16)	-----	5(17)	2(13)	4(25)	1(20)	3(11)	1(10)	19(18)	7(9)
Aug. 3-9	1(9)	4(11)	3(16)	--------	3(10)	2(13)	3(19)	--------	6(21)	1(10)	16(16)	7(9)
Aug. 10-16	--------	6(17)	5(26)	--------	4(14)	--------	1(6)	--------	7(25)	3(30)	17(17)	9(12)
Aug. 17-23	--------	2(6)	3(16)	1(8)	6(21)	--------	--------	1(20)	3(11)	1(10)	12(12)	5(6)
Aug. 24-30	--------	5(14)	1(5)	--------	--------	2(13)	1(6)	--------	--------	--------	2(2)	7(9)
Aug. 31-Sept. 6	--------	1(3)	2(11)	1(8)	1(3)	--------	--------	--------	1(4)	--------	4(4)	2(3)
Sept. 7-13	--------	2(6)	--------	1(8)	1(3)	--------	--------	--------	2(7)	--------	3(3)	3(4)
Total	11	35	19	12	29	15	16	5	28	10	103	77

[1] Chipmunk Creek only. [2] Grouse Creek only. [3] Chipmunk and Grouse upstream and downstream tagging combined.

Appendix table 7.—*Period fishing returns, all tagging areas, 1949 to 1953*

Time period	Arnica Creek 1950-53				Pelican Creek 1949-53				Chipmunk and Grouse Creeks 1949-53				Yellowstone Cascades 1951-53			
	First-year returns		Second-year returns		First-year returns		Second-year returns		First-year returns		Second-year returns		First-year returns		Second-year returns	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
OPENING OF LAKE FISHERY																
June 1-7	------	------	------	------	1	0.3	3	3	------	------	1	1	3	1	------	------
June 8-14	10	4	3	6	6	2	2	2	1	1	4	5	1	0.4	1	2
June 15-21	23	10	2	4	6	2	7	7	------	------	5	6	7	3	3	6
June 22-28	23	10	3	6	11	3	5	5	1	1	4	5	15	6	3	6
OPENING OF RIVER FISHERY																
June 29-July 5	38	16	8	16	37	10	14	15	2	2	7	9	63	26	14	27
July 6-12	29	12	3	6	48	13	14	15	4	4	7	9	55	23	11	22
July 13-19	28	12	3	6	38	10	11	11	7	7	5	6	43	18	8	16
July 20-26	27	11	10	20	78	21	15	16	15	15	4	5	28	12	3	6
July 27-Aug. 2	17	7	7	14	52	14	11	11	19	18	7	9	11	5	3	6
Aug. 3-9	17	7	5	10	35	10	5	5	16	16	7	9	4	2	3	6
Aug. 10-16	12	5	3	6	26	7	4	4	17	17	9	12	3	1	------	------
Aug. 17-23	6	3	------	------	13	4	2	2	12	12	5	6	3	1	------	------
Aug. 24-30	3	1	1	2	13	4	3	3	2	2	7	9	------	------	2	4
Aug. 31-Sept. 6	2	1	------	------	------	------	------	------	4	4	2	3	1	0.4	------	------
Aug. 7-13	------	------	1	2	------	------	------	------	3	3	3	4	1	0.4	------	------
Total	235	------	49	------	364	------	96	------	103	------	77	------	238	------	51	------

APPENDIX TABLE 7.—*Period fishing returns, all tagging areas, 1949 to 1953*—Continued

Time period	Trap Net 1952-53				Clear Creek 1953				Total for all locations			
	First-year returns		Second-year returns		First-year returns		Second-year returns		First-year returns		Second-year returns	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
OPENING OF LAKE FISHERY												
June 1-7									4	0.4	4	1
June 8-14									18	2	10	3
June 15-21			4	13	1	2			37	3	21	7
June 22-28	8	6	1	3	1	2	2	13	59	5	18	6
OPENING OF RIVER FISHERY												
June 29-July 5	38	29	10	32	1	2	1	7	179	16	54	17
July 6-12	34	26	7	23	4	9			174	16	42	13
July 13-19	29	22	4	13	4	9	3	20	149	13	34	11
July 20-26	10	8	3	10	8	19	4	27	166	15	39	12
July 27- Aug. 2	6	5	1	3	7	16	1	7	112	10	30	9
Aug. 3-9	3	2			10	23	4	27	85	8	24	8
Aug. 10-16	1	1	1	3					59	5	17	5
Aug. 17-23					6	14			40	4	7	2
Aug. 24-30					1	2			19	2	13	4
Aug. 31-Sept. 6									7	1	2	1
Aug. 7-13									4	.4	4	1
Total	129		31		43		15		1,112		319	

APPENDIX TABLE 8.—*Fishing rates and period fishing returns obtained from trap-net tagging, 1952 and 1953*

Time period	1952				1953			
	Period tag returns		Cumulative availability (number)	Rate of fishing (percent)	Period tag returns		Cumulative availability (number)	Rate of fishing (percent)
	Number	Percent			Number	Percent		
OPENING OF LAKE FISHERY								
June 1-7								
June 8-14								
June 15-21							6	0.0
June 22-28	5	8	111	4.5	3	4	103	2.9
OPENING OF RIVER FISHERY								
June 29-July 5	11	18	156	7.1	27	40	192	14.1
July 6-12	17	28	215	7.9	17	25	216	7.9
July 13-19	15	25	284	5.3	14	21	215	6.5
July 20-26	6	10	305	2.0	4	6	211	1.9
July 27-Aug. 2	4	7	299	1.3	2	3	210	1.0
Aug. 3-9	2	3	295	.7	1	1	208	.5
Aug. 10-16	1	1	293	.3			207	.0
Aug. 17-23			292	.0			207	.0
Aug. 24-30			292	.0			207	.0
Aug. 31-Sept. 6			292	.0			207	.0
Sept. 7-13			292	.0			207	.0
Total	61		292		68		207	

APPENDIX TABLE 9.—*Fishing mortalities obtained from trap-net tagging, 1952 and 1953*

[Numbers in parentheses indicate numbers of immature fish]

Time period	1952			1953		
	Tag group (number)	Group returns (number)	Fishing mortality (percent)	Tag group (number)	Group returns (number)	Fishing mortality (percent)
OPENING OF LAKE FISHERY						
June 1-7	------	------	------	------	------	------
June 8-14	------	------	------	------	------	------
June 15-21	4(6)	0(0)	0	6	2	33
June 22-28	107(18)	27(0)	25	97	30	31
OPENING OF RIVER FISHERY						
June 29-July 5	50(18)	14(0)	28	92	26	28
July 6-12	70(25)	15(1)	21	51	7	14
July 13-19	86(22)	2(1)	2	16	2	13
July 20-26	36(12)	3(0)	8	10	1	10
July 27-Aug. 2	------	------	------	3	------	------
Aug. 3-9	------	------	------	------	------	------
Aug. 10-16	------	------	------	------	------	------
Aug. 17-23	------	------	------	------	------	------
Aug. 24-30	------	------	------	------	------	------
Aug. 31-Sept. 6	------	------	------	------	------	------
Aug. 7-13	------	------	------	------	------	------
Total	353(101)	61(2)	17.3(2.0)	275	68	24.7

APPENDIX TABLE 10.—*Fishing rates and period fishing returns obtained from tagging at the Cascades of the Yellowstone River, 1951 to 1953*

Time period	1951				1952				1953			
	Period tag returns		Cumulative availability (number)	Rate of fishing (percent)	Period tag returns		Cumulative availability (number)	Rate of fishing (percent)	Period tag returns		Cumulative availability (number)	Rate of fishing (percent)
	Number	Percent			Number	Percent			Number	Percent		
OPENING OF LAKE FISHERY												
June 1-7	------	------	------	------	3	4	110	2.7	------	------	------	------
June 8-14	------	------	76	0.0	1	1	223	0.4	------	------	------	------
June 15-21	2	2	126	1.6	5	7	318	1.6	------	------	77	0.0
June 22-28	2	2	181	1.1	7	10	386	1.8	6	8	176	3.4
OPENING OF RIVER FISHERY												
June 29-July 5	19	21	179	10.6	22	31	384	5.7	22	28	256	8.6
July 6-12	22	25	332	6.6	15	21	362	4.1	18	23	343	5.2
July 13-19	25	28	354	7.1	4	6	347	1.2	14	18	331	4.2
July 20-26	11	13	329	3.3	7	10	343	2.0	10	13	319	3.1
July 27-Aug. 2	6	7	318	1.9	2	3	336	0.6	3	4	309	1.0
Aug. 3-9	1	1	312	0.3	1	1	334	0.3	2	3	306	0.7
Aug. 10-16	1	1	311	0.3	1	1	333	0.3	1	1	304	0.3
Aug. 17-23	------	------	310	0.0	1	1	332	0.3	2	3	303	0.7
Aug. 24-30	------	------	310	0.0	------	------	331	0.0	------	------	301	0.0
Aug. 31-Sept. 6	------	------	310	0.0	1	1	331	0.3	------	------	301	0.0
Sept. 7-13	------	------	310	0.0	1	1	330	0.3	------	------	301	0.0
Total	89	------	310	------	71	------	329	------	78	------	301	------

APPENDIX TABLE 11.—*Fishing mortalities obtained from tagging at the Cascades of Yellowstone River, 1951 to 1953*

Time period	1951			1952			1953		
	Tag group (Number)	Group returns (Number)	Fishing mortality (Percent)	Tag group (Number)	Group returns (Number)	Fishing mortality (Percent)	Tag group (Number)	Group returns (Number)	Fishing mortality (Percent)
May 25-31	---	---	---	38	7	18	---	---	---
OPENING OF LAKE FISHERY									
June 1-7	---	---	---	72	14	19	1	0	0
June 8-14	76	12	16	116	20	17	30	5	17
June 15-21	50	11	22	96	17	18	46	4	9
June 22-28	57	19	33	73	12	16	99	27	27
OPENING OF RIVER FISHERY									
June 29-July 5	---	---	---	5	1	20	86	17	20
July 6-12	172	38	22	---	---	---	109	24	22
July 13-19	44	9	20	---	---	---	6	1	17
July 20-26	---	---	---	---	---	---	2	0	0
July 27-Aug. 2	---	---	---	---	---	---	---	---	---
Aug. 3-9	---	---	---	---	---	---	---	---	---
Aug. 10-16	---	---	---	---	---	---	---	---	---
Aug. 17-23	---	---	---	---	---	---	---	---	---
Aug. 24-30	---	---	---	---	---	---	---	---	---
Aug. 31-Sept. 6	---	---	---	---	---	---	---	---	---
Sept. 7-13	---	---	---	---	---	---	---	---	---
Total	399	89	22.3	400	71	17.8	379	78	20.6

APPENDIX TABLE 12.—*Fishery returns, by areas, from stream tagging*

Stream and year tagged	Returns from Yellowstone River area				Returns from Fishing Bridge area				Returns from Northern area			
	Number of first-year returns	Percent of total for year	Number of second-year returns	Percent of total for year	Number of first-year returns	Percent of total for year	Number of second-year returns	Percent of total for year	Number of first-year returns	Percent of total for year	Number of second-year returns	Percent of total for year
Arnica Creek:												
1950	2	4.5	0	0	4	9.1	1	7.7	10	22.7	2	15.4
1951	2	2.5	0	0	7	8.7	0	0	10	12.3	6	50.0
1952	0	0	0	0	2	5.7	0	0	8	22.8	1	50.0
1953	2	2.9	0	0	0	0	1	6.7	10	14.5	3	20.0
Total	6	2.7	0	0	13	5.7	2	4.8	38	16.5	12	28.6
Pelican Creek:												
1949	0	0	0	0	8	30.8	3	21.4	12	46.1	10	71.4
1950	3	5.6	0	0	20	37.0	1	11.1	31	57.4	5	55.6
1951	2	2.9	0	0	8	11.4	7	29.2	52	74.3	15	62.5
1952	3	3.5	0	0	23	27.1	0	0	55	64.7	12	92.3
1953	1	1.0	1	4.3	20	19.2	2	8.7	69	66.4	12	52.2
Total	9	2.7	1	1.2	79	23.3	13	15.7	219	64.6	54	65.1
Clear Creek:												
1953	0	0	0	0	5	11.6	0	0	27	62.8	6	54.5
Chipmunk and Grouse Creeks:												
1949	0	0	0	0	0	0	0	0	1	9.1	8	23.5
1950	1	5.9	0	0	0	0	0	0	4	23.5	4	33.3
1951	0	0	0	0	0	0	0	0	9	32.2	0	0
1952	0	0	1	20.0	0	0	0	0	6	50.0	1	20.0
1953	0	0	0	0	0	0	0	0	4	19.0	1	20.0
Total	1	1.1	1	1.4	0	0	0	0	24	27.0	14	19.7

APPENDIX TABLE 12.—*Fishery returns, by areas, from stream tagging*—Continued

Stream and year tagged	Returns from Southern area				Returns from West Thumb area				Total returns for year	
	Number of first-year returns	Percent of total for year	Number of second-year returns	Percent of total for year	Number of first-year returns	Percent of total for year	Number of second-year returns	Percent of total for year	Number of first-year returns	Number of second-year returns
Arnica Creek:										
1950	2	4.5	1	7.7	26	59.2	9	69.2	44	13
1951	0	0	2	16.7	62	76.5	4	33.3	81	12
1952	1	2.9	0	0	24	68.6	1	50.0	35	2
1953	5	7.1	1	6.7	52	75.4	10	66.7	69	15
Total	8	3.5	4	9.5	164	71.6	24	57.1	229	42
Pelican Creek:										
1949	2	7.7	0	0	4	15.4	1	7.2	26	14
1950	0	0	1	11.1	0	0	2	22.2	54	9
1951	3	4.3	0	0	5	7.1	2	8.3	70	24
1952	3	3.5	0	0	1	1.2	1	7.7	85	13
1953	7	6.7	3	13.1	7	6.7	5	21.7	104	23
Total	15	4.4	4	4.8	17	5.0	11	13.2	339	83
Clear Creek:										
1953	5	11.6	2	18.2	6	14.0	3	27.3	43	11
Chipmunk and Grouse Creeks:										
1949	3	27.3	6	17.7	7	63.6	20	58.8	11	34
1950	7	41.2	5	41.7	5	29.4	3	25.0	17	12
1951	13	46.4	11	73.3	6	21.4	4	26.7	28	15
1952	4	33.3	2	40.0	2	16.7	1	20.0	12	5
1953	13	62.0	3	60.0	4	19.0	1	20.0	21	5
Total	40	44.9	27	38.0	24	27.0	29	40.9	89	71

LIMNOLOGY OF YELLOWSTONE LAKE IN RELATION TO THE CUTTHROAT TROUT

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LIMNOLOGY OF YELLOWSTONE LAKE IN RELATION TO THE CUTTHROAT TROUT

Norman G. Benson
Fishery Research Biologist

RESEARCH REPORT 56

UNITED STATES DEPARTMENT OF THE INTERIOR
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ABSTRACT

Limnological data collected from 1954 to 1959 on surface currents, bottom currents, temperatures, bottom soils, water chemistry, plankton, bottom fauna, and higher aquatic plants are related to the biology of the cutthroat trout, *Salmo clarki lewisi*, in Yellowstone Lake (Wyoming). The lake, formed on Eocene lava, is oligotrophic and low in dissolved solids. *Diaptomus shoshone*, *Daphnia shoedleri*, and *Conochilus unicornis* made up more than 90 percent of the macroscopic zooplankton by number. Upwelling in West Thumb was demonstrated by temperature stratification and conductivities. Plankton distribution and abundance were related to currents and water chemistry. Depth distribution, feeding, and movements of trout are related to food abundance. Trout less than 315 millimeters in total length are not caught readily because of their feeding habits and greater dispersal. There is evidence that the heavy trout harvest in the northern part of the lake allowed *Gammarus lacustris*, an important trout food, to become locally abundant.

LIMNOLOGY OF YELLOWSTONE LAKE IN RELATION TO THE CUTTHROAT TROUT

By Norman G. Benson, *Fishery Research Biologist*

Rocky Mountain Sport Fishery Investigations
Logan, Utah

Limnological studies on Yellowstone Lake (Wyoming) began in 1890 when Forbes (1893) described the common zooplankters, noted their relative abundance, and recorded other general features of the lake. Except for occasional records of water temperatures and a few plankton collections, the lake was not studied limnologically again until 1954, when the staff of the Rocky Mountain Sport Fishery Investigations initiated the work reported here.

Much has been published on the biology of the cutthroat trout in Yellowstone Lake and tributaries (Cope, 1956, 1957; Ball, 1955; Laakso and Cope, 1956; Ball and Cope, 1961). The purpose of this paper is to describe general features of the lake ecology and to relate this information to the known life history of the cutthroat trout.

Orville Ball, Martin Laakso, and several fishery aids mapped the lake and collected some field data. Identification of zooplankters and bottom organisms was by Marvin Meyer, Alan Stone, W. W. Wirth, A. W. Bell, and Clarence Shoemaker; Arthur Holmgren identified the higher aquatic plants. I appreciate the critical review of the manuscript by Oliver B. Cope, C. J. D. Brown, W. T. Edmundson, and Ross V. Bulkley.

The fish fauna of Yellowstone Lake includes the cutthroat trout, the longnose sucker *Catostomus catostomus* (Forster), the longnose dace *Rhinichthys cataractae* Valenciennes, the smallfin redside shiner *Richardsonius balteatus hydrophlox* (Cope), and the lake chub *Hybopsis plumbea* Agassiz. Only cutthroat trout and longnose suckers are found throughout the lake. The other species are restricted to shallow lagoons in the northern end and West Thumb (fig. 1), to very shallow (less than 2 meters deep) protected littoral zones of the lake, or to tributary streams. The original fish fauna included only the cutthroat trout and the longnose dace.

Information is presented on lake geology, lake morphometry, weather, water levels, bottom currents, surface currents, temperatures, bottom soils, water chemistry, phytoplankton, zooplankton, food of trout, and depth distribution of trout. A discussion of the relations of these conditions to the biology of the cutthroat trout in the lake follows.

GEOLOGY

The watershed of Yellowstone Lake has been an area of volcanic activity since the Eocene period, when large amounts of volcanic breccias were deposited (Bauer, 1955). With another heavy breccia deposit in the middle Cenozoic era, the whole series of breccias formed a stratum about 2,000 meters thick. Rhyolite deposits were superimposed on the breccias in certain areas in the late Cenozoic. The original breccias are exposed on the southern and eastern drainage basins of Yellowstone Lake, and rhyolite deposits are exposed on the south shore and in the western drainage area. Small deposits of dolomite, basalt, and glacial drift are present in isolated sections of the watershed.

The available information does not allow an adequate explanation of the geological formation of the present basin of Yellowstone Lake, although the original lake basin was believed to have been formed by a subsidence or readjustment of the original lava flows in the Eocene. Bauer also mentions the possibility that pre-Eocene glaciation may have formed the original basin. Recent glaciation, erosion, and milder forms of volcanic activity have sculptured the present basin. Many active geysers and hot springs are in the watershed. The West Thumb area is considered to be an old geyser basin, and hot springs are still present on the shoreline and below the water surface.

During its geological history, the lake has drained into three oceans. Until the early Pleistocene, the lake drained into the Pacific Ocean via the Snake River. At that time the Grand Canyon of the Yellowstone formed, and the lake drained northward into Hudson Bay. Since the retreat of the glaciers, the lake has drained through the Missouri River system into the Gulf of Mexico. During the glacial period, the lake was much larger than at present, owing to a glacial lobe at the present site of the Grand Canyon of the Yellowstone (fig. 1). Several lake terraces from the glacial period can be observed in Hayden Valley. One lake terrace has a present elevation of 2,470 meters, or 112 meters higher than the lake altitude.

WEATHER AND WATER LEVELS

The lake watershed is on the east slope of the Continental Divide at an altitude of 2,358 meters (7,731 feet). Air temperatures, wind measurements, and precipitation for the Yellowstone Lake area have been collected at the Lake Ranger Station for the U.S. Weather Bureau. The mean monthly temperatures from 1953 to 1957 ranged from minus 15.3° C. (4.5° F.) in February 1956, to 16° C. (60.8° F.) in July 1954. The mean monthly temperature during most summer months averaged about 13° C. (55.4° F.), and during winter months averaged minus 9.5° C. (14.9° F.). Air temperatures in the summer rarely reach 30° C. (86.0° F.). The annual precipitation from January 1953 to November 1957 varied from 39.3 cm. (15.49 inches) in 1953 to 62.2 cm. (24.47 inches) in 1955. Most precipitation falls during the late fall and winter months in the form of snow.

Data collected at the Lake Ranger Station on wind velocity and direction showed that the predominant winds were from the south and southwest (table 1), and that wind velocities were higher when the wind came from the latter two directions. Winds were stronger in 1955, 1956, and 1959 than in 1957 and 1958. Diurnal

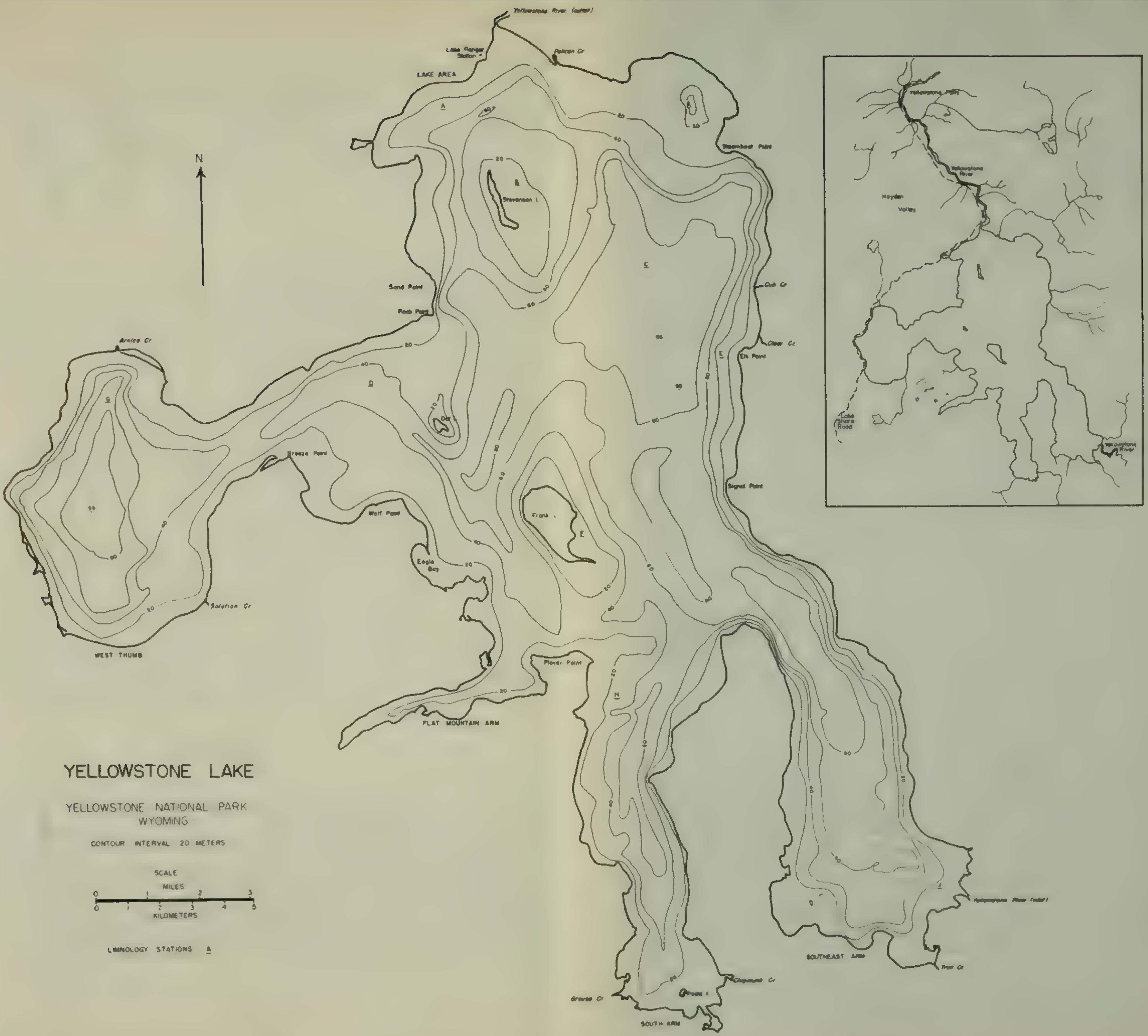

FIGURE 1.—Contour map of Yellowstone Lake showing limnology stations and localities mentioned in the report.

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TABLE 1.—*Wind velocity and direction at Lake Yellowstone during July and August from 1955 to 1959*

[Data collected daily at 4 p.m., at Lake Ranger Station]

Year	Mean daily velocity in miles per hour	Days recorded from—							Days with no wind recorded
		S.	SW.	SE.	E.	N.	W.	Other	
1955	5.92	25	17	4	1	0	10	0	5
1956	7.04	24	18	1	2	4	6	7	0
1957	1.35	21	4	1	4	2	0	2	28
1958	2.30	23	6	8	5	2	3	8	7
1959	8.05	28	18	2	0	5	5	4	0

changes in wind intensity were quite regular in that the high velocity winds usually began about 11 a.m. and decreased about 5 p.m. Calm conditions usually prevailed during the evenings and early morning. The highest air temperatures prevailed during the period of maximum wind velocity and the resultant wave action. This daily cycle undoubtedly increased the warming effects of the sun on water temperatures, since surface temperatures sometimes increased 3° C. from early morning to the time of maximum wind intensity.

Information on water levels and volume of outflow has been collected by the Surface Water Branch of the U.S. Geological Survey (1952, 1953, 1954, 1955; also unpublished data). The annual water level fluctuation of Lake Yellowstone varies from 1.5 to 1.8 meters. The water level is lowest from January to March, rises rapidly to a high in late June and July, and recedes from July to December. The annual outflow in thousands of cubic meters through the Yellowstone River varied by water year, October through September, as follows: 1951–52, 1,386,454; 1952–53, 1,270,505; 1953–54, 1,284,073; 1954–55, 1,003,674; 1955–56, 1,587,798; 1956–57, 1,270,949; and 1957–58, 939,482. The variation in outflow by month follows the water-level curve described above, with a maximum in July and a minimum in December. The capacity of Yellowstone Lake is about 14×10^9 cubic meters. The evaporation rate is about 358,948,500 cubic meters per year, considering an approximate annual evaporation rate of 1.016 meters (derived from p. 151 in Wisler and Brater, 1949). Thus, lake water is completely replaced about every 8 to 10 years.

Lake Yellowstone freezes over in December or January, and ice does not leave until the last week of May or early June. The ice reaches a thickness of 0.4 to 0.9 meters.

MORPHOMETRY

The bottom configuration of Yellowstone Lake was mapped with a fathometer mounted on a launch during the summers of 1954, 1955, and 1956. The depth contours were measured by a series of transects at 1-mile intervals in both north-south and east-west directions throughout the lake. Transects were made in other directions to verify the depths determined by the grid method and to measure various arms. A map was constructed to 20-meter contours (fig. 1).

Yellowstone Lake has a surface area of 35,391 hectares (136.66 square miles), a maximum measured depth of 98 meters (320 feet), a mean depth of 42 meters (139 feet), and a basin capacity of 14×10^9 cubic meters (12,095,264 acre feet). The areas of lake bottom at various depth ranges are shown in table 2. The lake basin has a deep trench along the east shore which extends into both the South Arm and Southeast Arm. It extends westward between Stevenson and Frank Islands to a point near the west shore of the main body of the lake. The lake basin in West Thumb is more cone-shaped, with no islands and with the deepest area in the approximate center of the thumb.

The lake is shaped like a hand with three fingers and a thumb, and has a shoreline development of 3.04. The watershed has an estimated area of 261,590 hectares (1,010 square miles) and consists principally of lodgepole pine forest and alpine meadows. The largest stream entering the lake is the upper Yellowstone River, but the lake cannot be considered simply an enlarged section of the river, since 42.2 percent of the lake watershed is outside the upper Yellowstone River drainage.

TABLE 2.—*Areas of various depth ranges in Yellowstone Lake*

Depth range	Area, in—			Percent of total
	Square miles	Acres	Hectares	
Less than 20 meters	31.44	20,121	8,143	23.01
20–40 meters	34.82	22,282	9,017	25.48
40–60 meters	26.70	17,088	6,916	19.54
60–80 meters	26.40	16,893	6,837	19.32
80–95 meters	17.29	11,066	4,478	12.65
Total area of lake	136.65	87,450	35,391	---------

METHODS

Limnological stations.—Originally, in 1954, 20 stations were established for measuring physical and biological conditions. In 1957 the number was reduced to 10 because it was found that variations in the lake could be accurately determined from the 10 stations shown on figure 1.

Temperature and surface currents.—Temperatures were measured by a bathyothermograph (depth range, 61 m.) which was calibrated periodically with a resistance thermometer. Temperatures below 61 meters were collected with a reversing thermometer. For describing water stratification, the metalimnion as defined by Hutchinson (1957) has been used to define the whole region in which the temperature gradient is steep.

Surface currents were determined by releasing 340 drift bottles on 16 different seeding lines in the lake. The bottles were designed by the Great Lakes Biological Laboratory (Johnson, 1958). These bottles were found to resist wind action unless surface currents were produced by wind. To facilitate recovery, our bottles were painted red by swirling thinned paint on the inside. The bottles were released while maintaining a launch at a constant speed and releasing the bottles at constant time intervals. Bottles were recovered, and their locations noted, by National Park Service employees, tourists, and U.S. Fish and Wildlife employees.

Bottom currents. — Bottom currents were measured by a modification of the leaning-tube-type current indicator described by Carruthers (1958a and 1958b). The indicator (fig. 2) proved accurate enough to measure current speeds to the nearest tenth of a knot and to measure direction to within 20 degrees. The graph illustrated by Carruthers (1958b) was used for recording current speed, since it was found that our modification of his bottle followed this curve the same as a bottle constructed to his specification.

Water chemistry.—Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (U.S. Public Health Association, 1955) was used as a guide for dissolved-oxygen, free-carbon-dioxide, and alkalinity determinations. Oxygen values were corrected for temper-

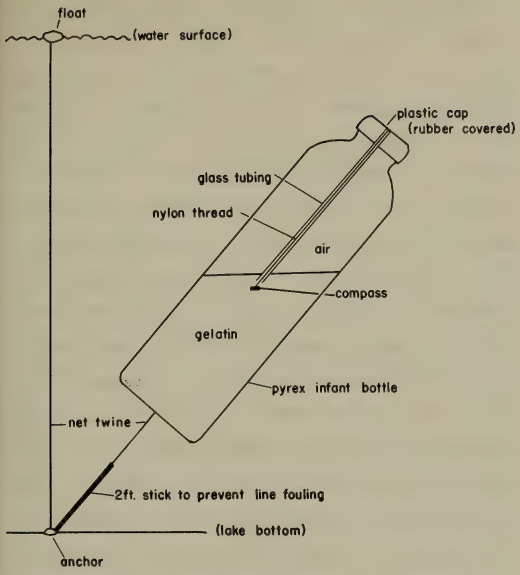

FIGURE 2.—Diagrammatic sketch of bottom-current indicator.

ature and altitude from the nomograph in Hutchinson (1957). Specific conductance was determined by a conductivity bridge and readings were converted to a standard 25° C. A more complete chemical analysis of four water samples was conducted by the Surface Water Branch of the U.S. Geological Survey.

Secchi disk transparency.—A Secchi disk (20 cm. in diameter, black and white) was used to measure the limit of visibility, and a water telescope was utilized to eliminate surface glare.

Bottom soils.—Five bottom-soil samples were analyzed for chemical properties by the Soils Laboratory of Utah State University. The percentage of organic matter in 59 additional samples was determined by the laboratory. Observations were made on the distribution of boulders, rubble, silt, sand, and other bottom types in different sections of the lake.

Plankton.—Net plankton tows were made with a Wisconsin plankton net using No. 25 bolting cloth during 1956. This method sampled the top 10-meter stratum of water and the net strained an estimated 211 liters (10.99 cu. ft.) of water. Several inherent errors in this method (as summarized by Welch, 1948) are recognized. The samples were preserved in a 3-percent solution of formaldehyde.

In 1957 and 1958 water for net plankton analyses was collected with a 3-liter Kemmerer water sampler. Each sample consisted of 10 liters of water and was strained through a plankton net with No. 25 bolting cloth. Plankton samples for phytoplankton measurements were counted in a Sedgewick-Rafter counting cell under a magnification of 100×. For zooplankton analyses, samples were counted with a rotary-type counter under a dissecting microscope at a magnification of 30× (Ward, 1955). Data on phytoplankters are weak because many forms pass through No. 25 bolting cloth. Only abundant zooplankters were identified to species.

Bottom organisms.—Bottom samples were collected with a 6- by 6-inch (231 sq. cm.) Ekman dredge, and the organisms were separated from the bottom soils by sieving through a No. 30 Tyler (0.589 mm.) screen. Four samples were collected at each station. Sandy or rocky bottoms could not be sampled adequately with the Ekman dredge, although we determined the type and relative abundance of organisms in these bottom types by taking several dredge samples. Common forms were identified to species by specialists.

Stomach analyses.—Stomachs for food contents were collected from anglers by creel-census clerks in 1957 and 1958. Additional stomachs were collected with gill nets in 1957 and 1958. All were preserved in formalin and sorted in the laboratory. Organisms were identified as closely as possible and counted. Aliquots were used in counting large samples.

Gill netting.—Experimental gill nets (125 by 6 ft.) with square meshes 2½, 2, 1½, 1, and ¾ inches were used for sampling fish in different sections of the lake at different depths. All nets were set from 3 to 6 p.m. on one day and picked up from 8 to 10 a.m. the following morning. Each set is considered to be an equal sample of an overnight set.

Areas of lake.—Three areas of the lake have been differentiated for purposes of comparing lake limnology with movements of trout and fishing pressure intensity. The West Thumb area consists of the lake west of a line between Rock Point and Wolf Point and includes 19.1 percent of the lake area (see fig. 1). The northern area consists of all of the lake north of a line between Rock Point and Elk Point and includes 25.4 percent of the area. The southern area consists of all the lake south and east of these lines and includes 55.5 percent of the area.

Catch of fish.—Creel census data were collected on Yellowstone Lake from 1950 to 1959 and the method has been described (Moore, et al., 1952). The catch each year was computed from sampling different segments of the fishery, such as shore fishermen, guide boats, and private boats. All of the fishing in each segment of the creel census was done predominantly in a certain area. It was thus possible to determine with some accuracy the total catch each year as coming from each of the three areas of Yellowstone Lake. The West Thumb harvest includes all rowboats and guide boats from West Thumb, 20 percent of the trailer-boat catch, and 25 percent of the shoreline catch. The catch from the southern area consisted of 75 percent of the cruiser catch, 15 percent of the trailer boat catch, and 5 percent of the catch from Lake or Fishing Bridge guide boats. All remaining fish caught were taken in the northern area.

LAKE CURRENTS

Surface currents. — Certain considerations are necessary in the interpretation of drift bottle data:

1. Few bottles were recovered in the open water, and the route that a bottle followed from its release point to its destination was unknown. The predominant currents in the lake were deduced from a series of recoveries.
2. The number of bottles recovered on certain isolated southern sections of the lake shore was low owing to infrequent visits by tourists and National Park Service employees. Special efforts were made by our employees to collect bottles in these areas, but many undoubtedly were lost.
3. Descriptions of recovery points were rarely precise and no attempts were made to interpret minor differences in the general current patterns from a few unusual recovery points.
4. Complete analyses of the influence of daily wind direction on currents is not possible. The wind data includes readings at 4 p.m. at the Lake Ranger Station and experience has shown that these readings did not always reflect conditions for the entire lake or even for a complete day.

Our surface-current information is based on the recovery of 155 bottles from 340 released, or a recovery rate of 45.6 percent. A summary of the release points and recovery points is plotted in appendix figures 1 and 2. Surface currents as derived from these recoveries are shown in figure 3.

The surface currents on Yellowstone Lake during 1957 and 1958 flowed mostly toward the north, east, or northeast, and were caused by the predominant winds from the south and southwest (table 1). Occasional winds from the east or southeast had much less velocity than winds from the south or southwest. Most recoveries were made on the north and east shores of the lake. The only area where the currents regularly deviated from the above-mentioned directions was around Frank Island and at the entrance to West Thumb. Several bottles were carried from seeding lines northwest of Frank Island (seeding lines *E* and *F* on appendix tables 1 and 2) into West Thumb and southeast of Frank Island. Most of the bottles that were released within West Thumb were collected on the north and northwest shores of the main body of the lake, pointing to the large amount of surface water leaving Thumb Lake. Surface water in the South and Southeast Arms also was carried northward, although many bottles were recovered on the east shores of these arms.

The current speed can only be approximated, but one bottle traveled a minimum of 7 miles in 1 day. Several bottles were collected after being carried 10 miles in 2 days.

Bottom currents.—Bottom currents were measured at 57 stations from August 20 to September 7, 1959, and the results plotted on figure 4. The following general conclusions were deduced from these measurements:

1. Some water flows along the lake bottom into West Thumb.
2. Currents southwest of Frank Island were variable, but flowed generally to the northeast.
3. Currents in the northeast main body of the lake flowed northward along the east shore and westward along the north shore.
4. There was evidence of clockwise bottom current circulation within West Thumb.

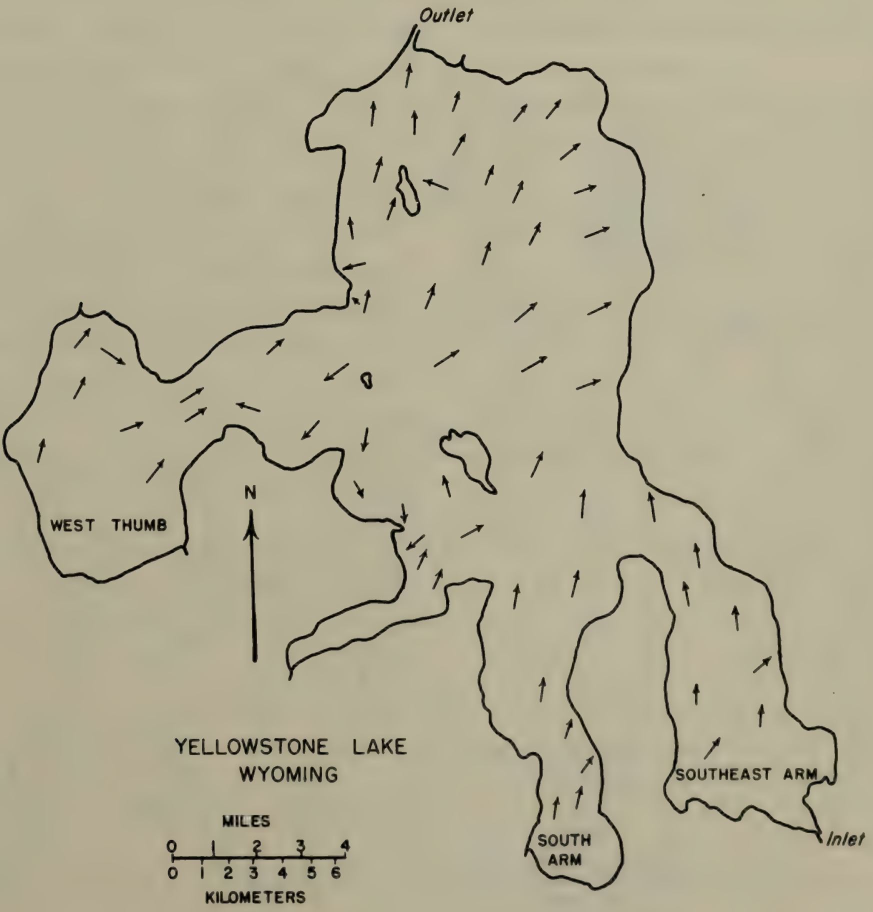

FIGURE 3.—Direction of surface currents on Yellowstone Lake as deduced from drift-bottle recoveries shown in appendix figures *A* and *B*.

5. No consistent relation was found between depth and current speed.

Relations between surface and bottom currents.—The predominant north and northeasterly flow of surface water in Yellowstone Lake is generally compensated by a northwesterly and southwesterly flow of water along the lake bottom. The great mass of water that piles up on the east shore of Yellowstone Lake forces bottom currents to flow in northerly and westerly directions, and bottom currents continue to flow westward back into West Thumb section. The bottom-current circulation in West Thumb suggests a clockwise movement. Mixture of bottom and surface water probably occurs in West Thumb as evidenced from our temperature and water-chemistry information presented later. Undoubtedly the subsurface current patterns described above are an oversimplification of the currents actually occurring in Yellowstone Lake, although our data do not allow a more complete description of the true conditions. Mortimer (1952) described the occurrences of internal seiches in Windemere, and both our current and temperature data (presented later), when compared with Mortimer's data, suggest similar conditions in Yellowstone Lake. This is true especially in the region from West Thumb to the northeast shore where the movement of surface water is most pronounced.

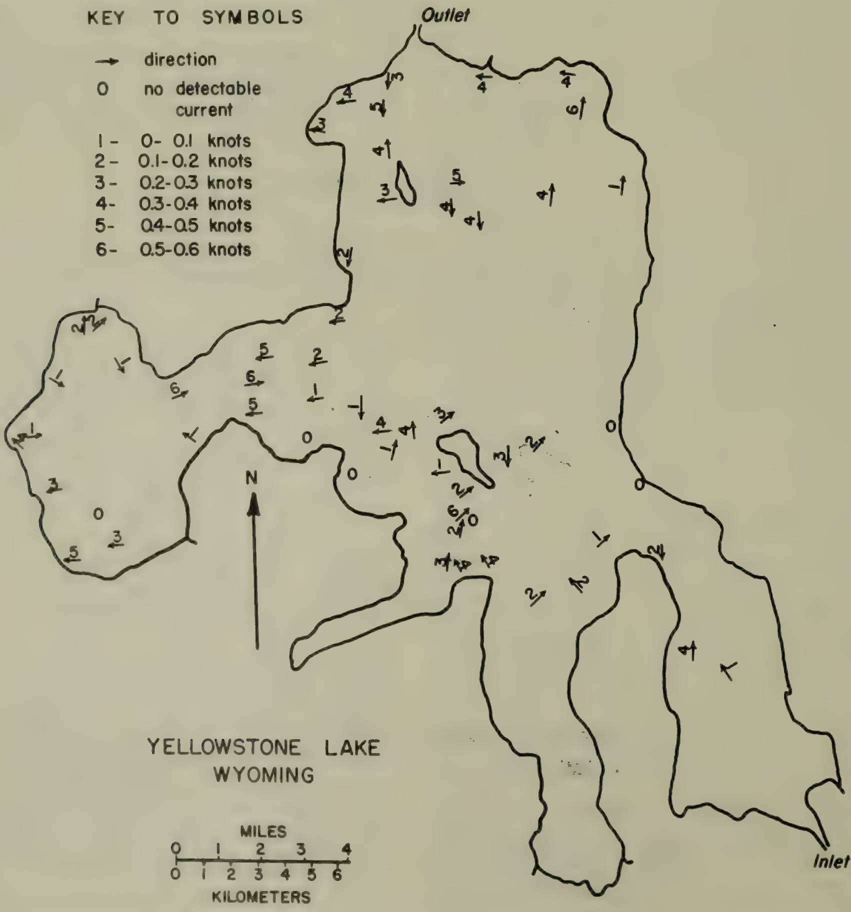

FIGURE 4.—Measurements of bottom currents in Yellowstone Lake from August 20 to September 7, 1959.

WATER TEMPERATURES

Water temperatures at different depths in Yellowstone Lake have been measured from June to the middle of October. Data from Station A have been used for illustrating seasonal and annual differences because it is north of the area of greatest surface water movement and the most complete data have been collected at this station (fig. 5). After the ice leaves near the end of May or in early June, the lake begins to warm, but remains virtually homothermous (4.5 to 5.8° C.) during early June. This period is comparable to the early spring warming period described by Church (1945) for Lake Michigan. In late June the surface water begins to form a protective warmer layer, although no clear-cut metalimnion has yet formed. At this time several temporary metalimnion layers have been observed (see June 30, 1959, in fig. 5), as has been recorded by Pennak (1955) from Grand Lake, Colorado. Surface temperatures reach between 10.0 and 14.0° C. during this phase.

In middle July a permanent metalimnion begins to form and the temperature gradient in the epilimnion narrows down to about 10° C. The depth of the epilimnion during its early formation usually varies from 5 to 10 meters. During late July and early August the stratification becomes complete and the summer stagnation period described by Church begins. Surface temperatures recorded during this period varied from 15.5 to 17.8° C., while the total temperature drop in the epilimnion was usually less than 4° C. The temperature gradient in the metalimnion varied from a drop of 12° C. in 10 meters to 10° C. in 3 meters. Temperatures below 90 meter depths during complete stratification were not recorded below 4.6° C., which is the temperature reached before stratification.

In late August the lake surface water begins to cool and the metalimnion starts to sink. This latter layer develops a smaller temperature range and the temperature gradient decreases. Temperatures in September at 30 meters were higher than in August, due to the sinking of the metalimnion. On October 15, 1958,

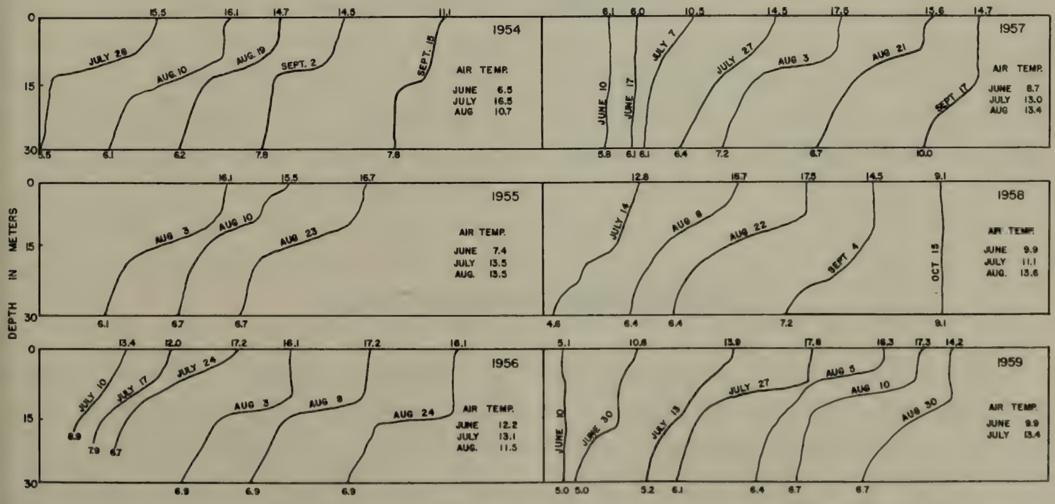

FIGURE 5.—Water temperatures (°C.) to 30 meters off Lake Area from 1954 to 1959. Mean monthly air temperatures each year included.

the surface temperature was 9.1° C. and a slight metalimnion was still present from 32 to 35 meters.

Differences in time of stratification among years appeared to be related to mean monthly air temperatures in June and July, shown on figure 5. Comparable data for 1956 and 1959 both showed earlier complete stratification than other years, and the mean air temperatures for these months were higher than recorded during other years.

A comparison of the water levels of Yellowstone Lake from 1954 to 1959 (table 3) with temperature stratification suggests that the thickest epilimnion strata formed in August during the years of high water such as 1954, 1956, and 1957. High water levels in 1959 were not, however, associated with a deep epilimnion. All years of high water showed a more abrupt temperature gradient in the metalimnion than the years of low water levels. This condition is possibly caused by the heavier stream runoff during high water level years, since stream temperatures reach either close to or above epilimnion temperatures soon after the first heavy runoff period in early July, and stream water, therefore, remains in and increases the depth of the epilimnion layer. The data suggest that Yellowstone Lake circulates twice a year and is a first-class dimictic lake, as classified by Hutchinson (1957).

Table 3.—*Mean monthly water levels in Yellowstone Lake during June, July, August, and September, 1954 to 1959*

[In meters above the datum of 2,357.48 meters. Date collected by U.S. Geological Survey, 1952, 1953, 1954, and 1955, and unpublished data]

Year	June	July	August	September
1954	1.31	1.58	1.03	0.64
1955	1.07	1.24	0.90	0.57
1956	1.83	1.59	1.03	0.66
1957	1.37	[1] 1.44	[1] 0.97	0.63
1958	1.07	1.07	[1] 0.75	[1] 0.50
1959		1.44	0.95	

[1] Data not complete.

Striking differences were evident between the thermal stratification patterns in the West Thumb area and in the east central part of the lake. West Thumb had a thin epilimnion and an indistinct metalimnion, while the north central section had a thick epilimnion and a very distinct metalimnion (fig. 6). Intermediate conditions existed at the neck of West Thumb and in other sections of the lake. These conditions were caused by the predominant winds from the south and southwest. Data from drift bottles showed that surface water is moved from West Thumb to the central and north central parts of the lake. This water movement probably caused an upwelling in West Thumb and a piling up of surface water in the eastern part of the lake. Miller (1952) and Ayers and associates (1958) observed conditions similar to this in certain windy areas of the Great Lakes.

SECCHI DISK TRANSPARENCY

Readings with a Secchi disk during August, 1957 and 1959, varied from 6.5 to 8.3 meters on overcast days and from 8.8 to 10.1 meters on clear days. Variations among different stations in the lake were not consistent and were not always related to plankton abundance. These readings were greater than those recorded by Pennak (1955) for mountain lakes in Colorado, even though all were collected during the August pulse of *Anabaena* or the period of lowest transparency.

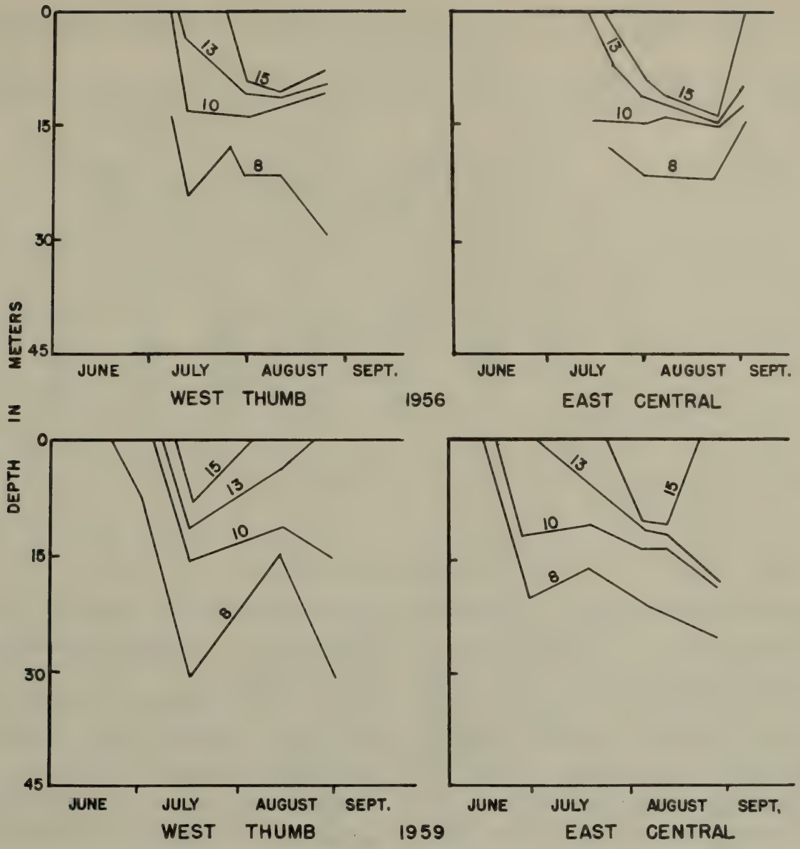

FIGURE 6.—Depths of isotherms (°C.) in West Thumb (station G) and the east central part of Yellowstone Lake (station C) during 1956 and 1959.

WATER CHEMISTRY

Yellowstone Lake is located approximately on the Continental Divide and drains a watershed of igneous rocks of volcanic origin. The chemical composition of the water is "primitive" in the sense that it has not been altered by civilization, and it does not drain sedimentary rocks.

Ionic composition.—The ionic composition of the water classifies it as the bicarbonate type, as defined by Rodhe (1949) (table 4). By arranging the major electrolytes into milliequivalents which presents their reacting weight, major anions exist in the order of HCO_3, SO_4, Cl. The major cations exist in the order Na, Ca, Mg, K, which is indicative of soft water draining igneous rocks. This ionic composition is similar to North German soft-water lakes summarized by Ohle (1955).

Bicarbonates ranged from 33 to 35 mg./l. while most ionic concentrations were relatively low. Several differences were apparent from a comparison of ions from the four sections of the lake. In general, West Thumb water had the highest values of Ca, Mg, Na, K, Cl, F, and B. Concentrations near the outlet and east of Stevenson Island were similar to each other and the lowest values were in the Southeast Arm. The higher values in West Thumb are due both to an upwelling

TABLE 4.—*Chemical analysis of water collected from 2 to 10 meters at four stations in Yellowstone Lake on July 28, 1958*

[Analyses by U.S. Geological Survey. Milliequivalents per liter (me./l.) presented for major cations and anions]

Constituents	Analysis of water collected in—							
	Middle Southeast Arm		Middle West Thumb		Four miles east of Stevenson Island		Near outlet	
	mg./l.	me./l.	mg./l.	me./l.	mg./l.	me./l.	mg./l.	me./l.
SiO_2	7.4	----------	7.2	----------	5.6	----------	5.7	----------
Fe	0.02	----------	0.01	----------	0.02	----------	0.02	----------
Ca	4.5	0.2245	5.0	0.2495	4.9	0.2445	4.5	0.2245
Mg	2.4	.1974	2.6	.2138	2.4	.1974	2.4	.1974
Na	8.0	.3478	9.4	.4087	9.1	.3957	8.9	.3870
K	1.8	.0460	2.0	.0512	1.5	.0384	1.7	.6435
HCO_3	33	.5408	35	.5737	35	.5737	34	.5573
SO_4	8.0	.1666	8.0	.1666	9.8	.2040	7.8	.1624
Cl	4.4	.1241	5.6	.1579	4.6	.1297	4.8	.1354
F	0.5	----------	0.6	----------	0.5	----------	0.5	----------
NO_3	0.2	----------	0.1	----------	0.1	----------	0.1	----------
B	0.08	----------	0.13	----------	0.09	----------	0.10	----------
Specific conductance (25° C.)	86.3	----------	96.4	----------	92.2	----------	93.2	----------
pH	6.8	----------	6.8	----------	6.7	----------	7.0	----------
Dissolved solids residue on evaporation at 180°C	54	----------	57	----------	55	----------	55	----------
Hardness	21	----------	23	----------	22	----------	21	----------

influence caused by south and southwesterly winds and possibly to hot spring activity on the west shore and below the water surface in West Thumb. Water from hot springs is rich in SiO_2, CO_2, Na, and Cl. Hot spring water would presumably rise to the surface immediately, and any influences would be recorded in our sampling. Upwelling water was considered to be of greater importance, however, because data presented later on conductivities indicated the increase in ionic concentrations was related to the clockwise circulation in West Thumb and not to the principal locality of hot spring activity in the extreme west end of the Thumb. Also, there is no evidence of local hot spring influence in the reading from the outlet where Pelican Creek contributes much water from hot springs. Higher SiO_2 in the Southeast Arm is believed to be due to the inflow of the Yellowstone River.

Conductivities.—Water conductivity is directly related to total dissolved solids or total ion concentration. Conductivities from various stations at 5 and 10 meters (epilimnion) in Yellowstone Lake showed that West Thumb usually showed the highest readings, while Southeast Arm showed the lowest readings (table 5). In general, the northern part of the lake showed higher readings than the southern section. The pattern of water circulation probably causes this condition. The monthly changes from July to September were not consistent at any station, although the highest epilimnion readings were usually recorded immediately after complete thermal stratification. In 1957 this occurred in early July and in 1959 it occurred in late June.

Conductivities usually increased with depth, but not consistently so (table 6). The stations which were most influenced by the summer water circulation, such as West Thumb, east of Dot Island, and Elk Point, showed maximum readings in mid-August. Those protected stations, such as east of Frank Island and in South Arm had minimum readings in August. The epilimnion layers were more shallow (6–8 m.) in the protected stations than in the exposed stations (12–15 m.), and it is reasonable to assume that a more concentrated trophogenic zone would use up nutrients more rapidly than a broad zone. Also, the mixture of surface and deep water in exposed stations would serve as a method of replenishment of nutrients.

TABLE 5.—*Conductivities (25° C.) in reciprocal megohms at different locations in Yellowstone Lake from 1957 to 1959*

[Dates of collection for each month below each year. Figures represent average of readings at 5- and 10-meter depths]

Location in lake	June		July			August			September			Means of readings by area
	1957 (June 20–25)	1959 (June 26–July 1)	1957 (July 2–10)	1958 (July 12–18)	1959 (July 14–22)	1957 (Aug. 19–23)	1958 (Aug. 6–14)	1959 (Aug. 10–13)	1957 (Sept. 15–18)	1958 (Sept. 3–4)	1959 (Aug. 30–Sept. 2)	
Off Lake area	101	--------	94	91	85	104	89	88	90	91	84	92
Five kilometers east of Stevenson Island	--------	84	103	86	91	101	89	57	63	91	92	86
Off Dot Island	95	87	115	90	91	103	99	98	105	89	88	96
Middle West Thumb	101	91	123	--------	83	99	--------	103	105	92	91	99
Off Frank Island	97	86	105	86	86	80	80	76	62	86	74	83
Mouth South Arm	101	86	101	--------	--------	78	93	76	64	86	75	84
Mouth Southeast Arm	85	--------	77	--------	72	75	86	97	63	--------	93	81
Mean readings for period	97	87	103	88	85	91	89	85	79	89	85	--------

TABLE 6.—*Variations in conductivities (25° C.), in reciprocal megohms, collected during 1959 by depth (meters) and location*

Location of collecting point	June 26–July 1					July 29–August 3					August 10–13						
	10	15	30	46	61	10	15	30	46	61	0	15	30	46	61	76	89
Middle West Thumb	91	91	93	100	92	89	89	93	96	97	----	103	104	107	106	----	----
East of Dot Island	88	96	90	94	95	86	85	89	88	----	----	98	108	105	98	103	----
Off Elk Point	91	88	87	90	88	75	73	----	----	----	95	93	95	96	91	89	89
Ten kilometers east of Stevenson Island	84	88	87	97	----	73	78	79	79	78	87	87	89	91	91	91	105
Mid South Arm	86	91	88	86	88	88	88	89	89	88	76	75	76	----	----	----	----
Five kilometers east of Frank Island	86	90	107	90	----	86	87	----	----	----	76	80	78	72	76	74	----
Mean by depth and sampling period	88	91	92	93	91	83	83	88	88	88	84	89	92	94	92	89	97

The distribution of conductivities by depth in and near West Thumb was measured on July 29, 1959, to determine how surface and bottom current measurements were related to conductivities (fig. 7). The data clearly demonstrated a clockwise circulation with conductivities increasing from the south to the north section of the area. The accumulation of additional nutrients from subsurface waters or upwelling can account for the increase in nutrients as water circulates in West Thumb. The hot spring activity is concentrated in the western part of the thumb. Both hot springs and upwelling probably accounted for the increases in the western and northern stations.

Alkalinity. — Bicarbonate alkalinities measured during 1957 ranged from 22 to 41 mg./1., usually varying from 30 to 33 mg./1. The readings showed the same general seasonal, horizontal, and vertical distributions as conductivities.

Dissolved oxygen.—Theinemann (1928) showed that lake morphometry has a strong bearing on oxygen concentrations, and that large, deep lakes rarely develop deficits. In 1957 oxygen was measured at all stations of the lake from mid-June to mid-September, and most readings down to 60 meters were near saturation. Some seasonal measurements of oxygen concentrations as compared with saturation values are shown in table 7, which also presents the lowest dissolved oxygen reading recorded, that of 53 percent saturation on July 25, 1957.

TABLE 7.—*Some summer dissolved oxygen concentrations (DO) from Yellowstone Lake during 1957 and 1959, compared with saturation (S) values*

[Values in milligrams per liter and adjusted for altitude. Collected at stations A and B]

Depth	June 20, 1957		July 25, 1957		Aug. 20, 1957		Sept. 18, 1957		Sept. 10, 1959	
	DO	S	DO	S	DO	S	DO	S	DO	S
0 meters	10.0	9.5	7.4	7.7	7.2	7.2	7.8	7.3	----------	----------
4.5 meters	10.0	9.5	7.4	7.8	7.2	7.3	8.2	7.5	----------	----------
9 meters	10.2	9.6	----------	----------	8.0	7.2	8.0	7.5	----------	----------
15 meters	----------	----------	----------	----------	----------	----------	----------	----------	8.5	8.8
20 meters	9.8	9.7	----------	----------	----------	----------	8.4	7.9	----------	----------
22 meters	----------	----------	8.3	8.6	8.3	8.3	----------	----------	8.3	9.2
30 meters	9.6	8.9	----------	----------	8.3	8.6	----------	----------	8.0	9.3
45 meters	----------	----------	4.9	9.2	8.3	8.7	----------	----------	8.7	9.2
60 meters	----------	----------	----------	----------	8.2	9.3	----------	----------	8.2	9.3
76 meters	----------	----------	----------	----------	----------	----------	----------	----------	8.0	9.5
92 meters	----------	----------	----------	----------	----------	----------	----------	----------	6.2	9.5

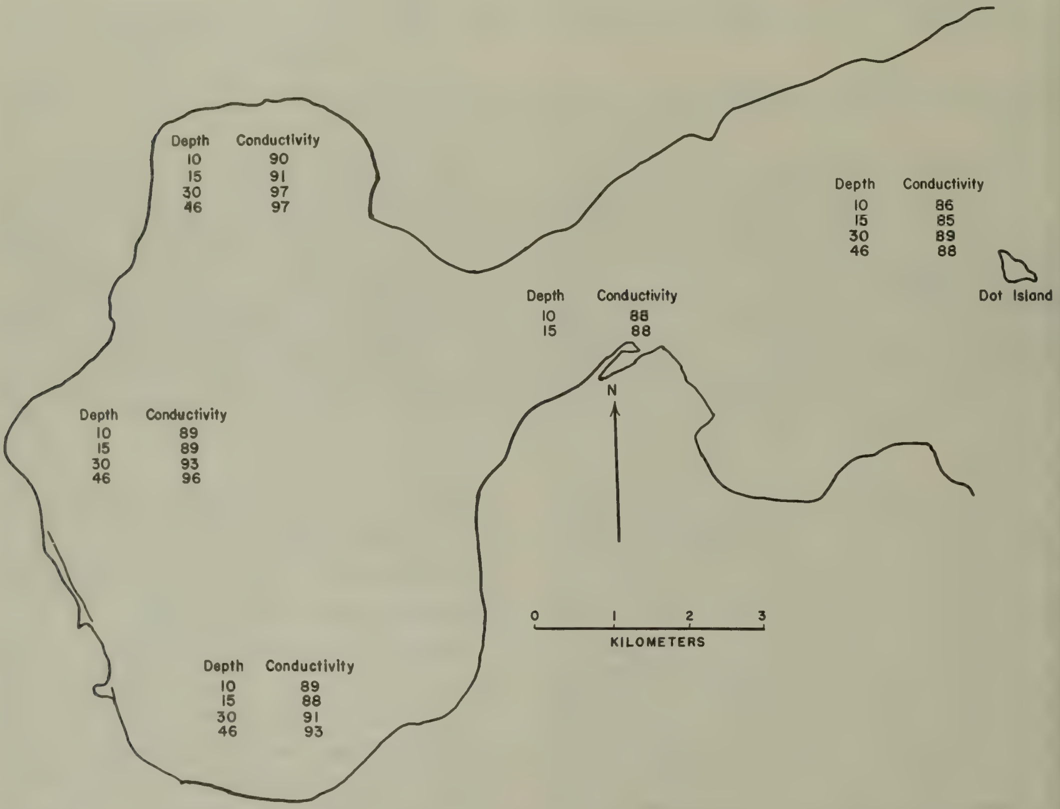

FIGURE 7.—Conductivities at different depths (meters) in West Thumb and near Dot Island on July 29, 1959.

Weather conditions in the summer of 1959 caused the water to stratify earlier than in 1957 or 1958 (fig. 5), which could presumably allow an oxygen deficit to increase. Measurements made on August 10, 1959, near Stevenson Island showed a deficit at 92 meters of 64 percent saturation. There is little question that during the short (approximately 45 days) period of complete stratification, a partial oxygen deficit develops below 30 meters in Yellowstone Lake. The relatively short period of complete stratification, however, prevents a severe depletion that would influence the composition of bottom fauna or would even prevent fish from inhabiting this zone. Pennak (1955) found a greater progressive oxygen deficiency during the summer by depth in Bear Lake, Colo., than was present in Yellowstone Lake. His data on phytoplankton and carbon dioxide, however, suggested higher biological activity for Bear Lake.

Free carbon dioxide.—Free carbon dioxide existed in all samples collected during 1957. Readings ranged from 1.5 p.p.m. on the surface to 13.3 p.p.m., at 61 meters (table 8). The water depth, which showed a pronounced increase in carbon-dioxide content (above 10 p.p.m.), decreased from 45 meters in July to 19 in August. The depth of 19 m. in August was just below the metalimnion.

TABLE 8.—*Free carbon dioxide concentrations in mg./l., by month and depth during summer of 1957*

[Mean of readings at various stations]

Depth	June	July	August	September
0 meters	3.5	3.5	----------	2.0
5 meters	3.8	4.3	3.5	3.4
10 meters	4.0	5.4	6.0	3.5
19 meters	----------	----------	12.5	4.0
30 meters	----------	3.0	----------	----------
45 meters	----------	11.5	12.8	----------
61 meters	----------	----------	13.3	----------

BOTTOM TYPES

Types and distribution.—Bottom types in Yellowstone Lake consist of boulders, rubble, black obsidian sand, and fine clays with organic matter. Boulders are exposed in quantity only on the east shore and on the windward shore of Frank Island. Rubble areas are concentrated on the east shore and in a few exposed sections of the South and Southeast Arms. The most common shallow (less than 2 m.) water bottom type is black obsidian sand which contains less than one percent organic matter. Silty loams with varying amounts of organic matter make up more than 95 percent of the bottom type of Yellowstone Lake. The amount of organic matter in the bottom sediments increases with depth, with a correlation coefficient from 59 samples of 0.80 (table 9). In general, bottom soils below 50 m. possess more than 5 percent organic matter, while bottom soils between 25 and 50 meters have more than 2 percent organic matter. There are many exceptions to this condition which can be explained by water movements. Areas with a greater movement or exchange of water, such as the Southeast Arm with the inflowing Yellowstone River, allow a smaller amount of organic matter to accumulate than do other similar areas, such as the South Arm, with less water exchange. Water currents, as described earlier, prevented organic matter from accumulating in as great a quantity in the neck of West Thumb as in areas of similar depth inside West Thumb. Greater than average amounts of organic matter accumulated around Sand Point, where an eddy current is probably present, as shown from several drift recoveries. Organic matter deposition on the lake bottom of less than 20 meters in depth has much influence on fish distribution and fish food production. Those areas with much organic matter had large stands of aquatic plants, which had high standing crops of tendipedids and *Gammarus*. As shown later, the latter organisms are important foods for cutthroat trout.

Chemical properties.—Bottom-sediment samples were analyzed to determine what differences in organic carbon, nitrogen, P_2O_5, and cation exchange existed in sections of the lake by depth. Soil samples were all classified as silt loam in agricultural terminology and were typical of the soft bottom sediments. The shallow stations had greater amounts of organic carbon and less phosphate (table 10) than the deeper stations. The cation exchange capacity was greater in the deeper stations. Nitrogen readings were not related to depth. The data show a greater utilization of nutrients in shallow water. With respect to growth conditions for aquatic plants, both Roelofs (1944) and Wohlschag (1950) found that organic matter content and site conditions were frequently of greater importance than phosphates, nitrates, or other nutrients, and these same factors appear to be most important in Yellowstone Lake.

Table 9.—*Percentage of organic matter in bottom soils by location and depth at 59 points in 13 sections of Yellowstone Lake*

Location and depth	Percentage of organic matter
West Thumb:	
25 meters	3.22
52 meters	4.13
70 meters	6.72
71 meters	6.33
75 meters	5.28
85 meters	6.14
88 meters	5.64
92 meters	5.81
94 meters	6.38
Neck of West Thumb:	
2 meters	0.31
13 meters	1.93
37 meters	1.72
Rock Point to Wolf Point:	
53 meters	1.75
22 meters	2.67
70 meters	7.43
64 meters	6.40
Mouth of Flat:	
33 meters	6.40
11 meters	1.48
Eagle Bay to Signal Point:	
6 meters	1.91
63 meters	6.02
12 meters	2.49
67 meters	4.45
75 meters	6.19
75 meters	6.23
Mouth of Southeast Arm:	
40 meters	4.20
69 meters	3.54
76 meters	3.23
49 meters	3.04
5 meters	1.22
South end of Southeast Arm:	
32 meters	4.64
40 meters	3.20
49 meters	3.58
37 meters	2.08
49 meters	1.94
1 meter	0.89
Mouth of South Arm:	
88 meters	3.54
53 meters	7.22
58 meters	6.69
South end of South Arm:	
15 meters	9.12
6 meters	6.40
1 meter	3.78
Frank Island to Elk Point:	
3 meters	0.22
61 meters	5.85
88 meters	5.42
88 meters	5.44
79 meters	5.85
Sand Point to Elk Point:	
92 meters	6.71
1 meter	3.66
79 meters	8.33
82 meters	5.90
82 meters	6.00
81 meters	6.24
46 meters	4.68
Stevenson Island to Steamboat Point:	
76 meters	6.43
85 meters	6.31
87 meters	6.88
Stevenson Island to outlet:	
31 meters	2.29
55 meters	6.72
53 meters	6.30

Table 10.—*Some chemical properties of six samples of bottom soils (silt loam) in Yellowstone Lake, collected August 15–30, 1958*

Location	Depth (meters)	pH	Organic carbon (percent)	Nitrogen (percent)	C/N ratio	P_2O_5 p.p.m.	Cation exchange capacity	Organic matter (percent)
Southeast Arm	40	6.5	2.60	.325	8.0	68	47.6	7.26
Southeast Arm	70	5.7	----------	.262	----------	118	58.0	----------
South Arm	15	6.8	3.70	.562	6.5	45	42.0	6.36
South Arm	3	6.0	3.38	.392	8.6	41	40.2	5.81
East of Stevenson Island	88	6.1	2.24	.415	5.3	280	54.0	3.85

AQUATIC PLANTS

The distribution and relative abundance of aquatic plants has a great bearing on the production of those bottom organisms which are important cutthroat trout foods. Collections of aquatic plants were made with a plant hook and while collecting bottom samples with an Ekman dredge. The species collected and their depth distributions were as follows:

	Meters
Potamogeton richardsonii	1– 5
Potamogeton praelongus	5–10
Potamogeton pusillus	1–18
Potamogeton robbinsii	5–10
Potamogeton gramineous var. *graminifolius*	0– 3
Lemna trisulca	2–18
Ceratophyllum demersum	1–17
Najas flexilis	2– 8
Myriophyllum exalbescens	1– 5
Ranunculus aquaticus	0.5– 5
Fontinalis sp.	2
Elodea canadensis	0– 2
Nitella flexilis	1
Eleocharis sp.	0.5– 1
Sagittaria cuneata	0.5
Sparganium sp.	0– 2

Aquatic plant beds were generally restricted to protected littoral areas where organic matter was allowed to accumulate and were not present below a depth of 18 meters. The most extensive beds were located at 1 to 10 meter depths in South and Southeast Arms, on the lee sides of Stevenson and Frank Islands, and near the outlet. On windy, exposed shorelines, such as on the east shore or on the windward side of Stevenson Island, plant beds were dense from 7 to 15 meters. Aquatic plants in West Thumb were more abundant on the protected south shore than on the exposed north shore.

PLANKTON

Only general statements can be made about the abundance, seasonal changes, and genera of phytoplankton from samples collected in 1956. Plankton collections in 1957 and 1958 were aimed primarily at sampling zooplankters.

Phytoplankton.—The siliceous nature of the watershed undoubtedly caused the predominance of diatoms in the phytoplankton. Except for an annual August pulse of *Anabaena*, various diatoms such as *Asterionella*, *Melosira*, and *Stephanodiscus*, dominated the phytoplankton flora (table 11). A dense pulse of *Asterionella* occurred in 1955, 1956, 1957, and 1958, soon after the ice left in early June. This pulse extended to 35 m. in depth and was associated with high water levels due to melting snows. *Melosira* formed a pulse to a lesser extent in late July and remained abundant until September. *Anabaena* formed a heavy pulse during the first two weeks of August during all years measured. This pulse caused a green cast in

TABLE 11.—*Mean number of phytoplankton cells collected per liter (to nearest whole number) from all sections of Yellowstone Lake, June 28 to August 31, 1956*

[Each sample represents one plankton haul from 10 meters to surface]

Alga genus	Number collected during period—								
	June 28–July 6 (10 samples)	July 10–17 (14 samples)	July 20–24 (13 samples)	July 30–31 (5 samples)	Aug. 1–3 (7 samples)	Aug. 7–10 (11 samples)	Aug. 15–16 (5 samples)	Aug. 20–24 (8 samples)	Aug. 29–31 (3 samples)
Asterionella	128, 711	115, 896	24, 764	54, 955	2, 349	961	1, 566	583	1, 073
Stephanodiscus	68	74	41	18	34	0	10	88	48
Melosira	2, 138	903	61	0	260	132	2, 125	48	128
Staurastrum	10	8	5	1	5	40	2	98	1
Coelastrum	0	14	14	0	12	4	1	20	82
Anabaena	889	123	591	7, 420	16, 807	11, 830	17, 998	389	0
Other [1]	1	11	11	1	3	2	4	33	6

[1] Includes *Coelosphaerium*, *Pleurococcus*, *Cosmarium*, *Navicula*, *Fragillaria*, *Ulothrix*, *Closterium*, *Pinnularia*, *Suriella*, *Compylodiscus*, unidentified desmids.

the water due to small clumps of algal filaments. Data from 1956 showed West Thumb to be the most productive section of the lake with Southeast Arm the least productive. Stations in the north and central areas of the lake were similar to those in South Arm and around Frank Island. These pulses were similar to those reported by Pennak (1955) for Grand Lake, Colo., although they were less intensive.

Zooplankton.—The zooplankton fauna consisted principally of *Conochilus unicornis*, *Diaptomus shoshone*, *Daphnia schoedleri*, and nauplii of *Diaptomus*. *Vorticella* occurred commonly during the *Anabaena* pulse in August, but no other species were commonly observed. A few *Cyclops* were identified, but they occurred so rarely (less than 0.1 percent) that they have been included with *Diaptomus* for purposes of enumeration. Also, a few *Diaptomus minutus* were identified. Forbes (1893) reported few *Cyclops*, but vast numbers of *Diaptomus*. *Keratella* was collected occasionally, but the rotifer fauna consisted almost entirely of *Conochilus unicornis*. Pennak (1957) found that one dominant species of copepod, cladoceran, or rotifer, was usually present in Colorado limnetic communities.

The vertical distributions of zooplankters showed that the depth range where the largest number occurred extended from 5 to 18 meters (table 12). *Diaptomus* were most abundant during July at 5 and 10 meters, but were present to 45 meters. Nauplii were most abundant during late July at the depth range of 15 to 23 meters. *Daphnia* were never abundant from any plankton samples, but increased in number in September. *Daphnia schoedleri*, the only cladoceran collected in Yellowstone Lake, is an inshore swarming species and was not sampled adequately at the

TABLE 12.—*Verical distribution of Diaptomus (Di), nauplii of Diaptomus (N), Daphnia (Da), and rotifers (R) during 1957 and 1958*

[Number per liter is derived from 10-liter samples combined from all limnological stations in Yellowstone Lake. Number of samples shown in parentheses. *Diaptomus* counts include a few *Cyclops*]

Depth and plankter	June 1–30	July 1–15	July 16–30	August 1–15	August 16–31	September 3–18	Mean number per sample
0 meters	(8)	(12)	(4)	(3)	(3)	(15)	(45)
Di	8.8	7.7	5.6	0.9	3.4	4.9	6.0
N	4.9	6.1	7.4	1.2	2.6	5.8	5.3
Da	0.2	0.1	0.1	0.3	0.1	2.3	0.9
R	0.2	0.5	37.2	0	5.5	2.4	4.6
5 meters	(7)	(11)	(8)	(11)	(12)	(16)	(65)
Di	10.5	18.7	21.7	13.7	13.2	14.9	15.4
N	8.7	11.8	10.2	10.9	12.0	13.8	11.6
Da	0.2	0.3	0.5	0.7	0.9	3.1	1.2
R	4.4	15.5	151.9	193.0	41.3	17.7	66.4
10 meters	(7)	(12)	(8)	(15)	(11)	(12)	(65)
Di	15.4	9.4	13.2	8.1	10.4	14.2	11.3
N	8.4	10.7	15.8	5.8	19.9	14.0	12.1
Da	0	0	0	1.7	0.8	3.5	1.2
R	1.2	17.2	85.0	61.5	51.2	22.5	40.8
15–18 meters	----------	(5)	(3)	(15)	(8)	(9)	(40)
Di	----------	3.2	5.1	3.4	5.0	11.7	5.7
N	----------	7.7	46.9	15.2	16.2	18.1	17.5
Da	----------	0	0.4	0.1	0.7	1.1	0.5
R	----------	0.3	168.0	53.2	59.1	10.6	46.8
21–23 meters	----------	----------	(1)	(6)	(1)	(2)	(10)
Di	----------	----------	3.5	2.1	2.7	8.9	3.7
N	----------	----------	55.7	8.0	17.7	15.0	15.1
Da	----------	----------	0	0	0	2.1	0.2
R	----------	----------	2.3	1.4	4.2	24.3	6.3
30 meters	----------	(2)	(2)	(6)	----------	(5)	(15)
Di	----------	10.5	4.3	1.2	----------	4.2	3.9
N	----------	11.0	17.4	2.1	----------	9.1	7.7
Da	----------	0	0	0.1	----------	0.6	0.2
R	----------	3.5	2.2	2.7	----------	2.0	2.5
45 meters	----------	(1)	----------	(1)	(1)	----------	(3)
Di	----------	8.0	----------	1.2	2.4	----------	3.9
N	----------	6.7	----------	4.1	0.7	----------	3.8
Da	----------	0	----------	0	0	----------	0
R	----------	0	----------	0.6	0	----------	0.2

various stations for this reason. In late August, 1959, two large swarms covering an estimated 14,000 cubic meters each were observed, and each swarm contained 400 to 500 *Daphnia* per liter. These concentrations were located by anglers who were taking advantage of the fish concentrations in these areas. The intensity of these swarms probably varies annually, since such large concentrations would probably have been observed previously. Tonolli (1954) found that annual cladoceran populations varied greatly in Italian alpine lakes due to meteorological conditions. He found that warmer weather increased populations significantly, and related these conditions to the velocity of cladoceran developmental processes. The concentration in 1959 may have been due to the fact that the epilimnion formed earlier than in other years observed (fig. 5). Rotifers were abundant from the middle of July to mid-August, and they extended in abundance down to 18 meters. Those plankton organisms important as food for cutthroat trout did not extend in abundance below 18 meters in depth.

The abundance of zooplankters amongst different sampling stations can be compared from our data on table 13. The Southeast Arm (Station J) showed the lowest numbers. This fact is probably due to the great exchange of water from the inflowing Yellowstone River and to the low amount of nutrients in this arm. *Diaptomus* and nauplii were never abundant in this section of the lake. The West Thumb section showed poor standing crops and this was due to the continual removal of surface water from this area as shown both by current and temperature information. The heaviest populations were in the northeast and central parts of the lake where surface water accumulates from wind action. McMahon (1954) found that the movement of surface water due to wind action also concentrated entomostracan plankters in the lee sections of Lakelse Lake, British Columbia, Canada. The extreme north section of the lake and the South Arm were similar in numbers collected. The large number at Station F on the lee side of Frank Island is believed to be due to its protected nature.

TABLE 13.—*Mean number of organisms per liter from June 15 to September 15, 1957, among 10 sampling stations in Yellowstone Lake*

[All samples collected at 5- and 10-meter depths and separated as *Diaptomus* (Di), nauplii of *Diaptomus* (N), *Daphnia* (Da), and rotifers (R). Sampling stations shown in figure 1]

Month and station	Number of samples	Di	N	Da	R
June:					
A	2	1.4	1.2	0	0
B	2	3.0	0.7	0	0
C	--------	--------	--------	--------	--------
D	--------	--------	--------	--------	--------
E	2	22.1	15.0	0	9.4
F	2	32.0	20.1	0.2	0
G	--------	--------	--------	--------	--------
H	2	11.7	11.4	0	0
I	2	20.0	14.4	0.5	0
J	2	0.3	0	0	0
July:					
A	4	16.3	10.2	0.2	39.1
B	2	22.5	13.3	0	7.0
C	2	21.2	21.6	0	353.6
D	2	21.6	14.3	0	10.1
E	2	20.5	34.3	0	57.7
F	3	19.5	6.3	0	253.0
G	2	12.9	10.8	0	71.4
H	4	22.5	12.8	0	42.1
I	4	18.8	17.4	0.7	53.0
J	4	8.7	4.4	1.2	55.5
August:					
A	4	13.0	8.8	0.8	119.4
B	4	25.7	15.3	1.6	84.1
C	4	13.7	15.3	5.8	61.8
D	4	13.4	14.7	0.1	166.2
E	3	13.7	12.7	0.7	80.5
F	4	15.8	13.7	1.1	170.8
G	2	10.4	6.6	0.9	36.5
H	4	11.4	24.4	0	117.1
I	4	8.8	14.8	0.5	36.6
J	4	4.6	9.4	2.1	50.9
September:					
A	2	6.1	8.5	1.4	39.3
B	1	3.2	6.8	0	22.8
C	1	15.1	8.0	3.7	0
D	2	5.6	3.7	0.9	9.3
E	--------	--------	--------	--------	--------
F	2	8.2	13.4	1.8	24.4
G	2	10.9	3.2	2.0	6.2
H	2	7.3	16.1	0.9	8.4
I	1	17.9	21.3	1.1	11.2
J	1	5.2	10.4	6.5	9.1

	Di	N	Da	R	All organisms
Mean, all samples:					
A	11.0	8.0	0.6	59.4	79.0
B	17.4	10.7	0.7	41.5	70.3
C	16.0	16.1	3.8	136.3	172.2
D	13.5	11.8	0.3	88.0	113.6
E	18.0	19.5	0.3	53.7	91.5
F	18.4	12.8	0.8	135.5	167.5
G	11.4	6.9	0.9	38.0	57.2
H	14.5	17.0	0.2	54.4	86.1
I	15.3	16.3	0.6	33.6	65.8
J	5.4	6.0	1.8	39.5	52.7

BENTHOS

Studies on benthos included the determination of vertical and horizontal distribution of the common organisms. The sampling of hard-botton areas of the littoral zone was sparse, but observation did not disclose any great concentrations of organisms in these areas, possibly because of heavy molar action. Undoubtedly, the numbers of Ephemeroptera and Trichoptera were much higher than our sampling disclosed. The common organisms present, their depth distribution, and abundance were as follows:

Lumbriculidae (usually *Limnodrilus* sp.)—sparsely present 0 to 95 meters on silty bottom.

Tubifex tubifex (O. F. Muller)—only occurred in abundance in clumps on delta of Yellowstone River in Southeast Arm—10 meters.

Helobdella stagnalis (Linnaeus)—sparsely distributed in entire lake down to 15 meters on silt bottom.

Hyallela azteca (Saussure)—common from 0 to 15 meters around aquatic plant bed.

Gammarus lacustris (Sars)—abundant on protected shores down to 30 meters—present to 43 meters—most abundant around aquatic plant beds.

Ephemerella sp.—common only on poorly sampled, rocky shorelines, from 0 to 8 meters.

Psychomyia sp.—rare from 1 to 5 meters.

Dicosmoecus sp.—common from 0 to 25 meters.

Tendipes (*Tendipes*)—abundant from 0 to 30 meters.

Tendipes (*Limnochironomus*) — moderately abundant to 90 meters.

Prodiamesa—present at all depths and most abundant from 70 to 90 meters.

Procladius—moderately abundant to 30 meters.

Pisidium—present from 0 to 30 meters.

Sphaerium—present from 20 to 60 meters.

The standing crops of benthic organisms at various depth ranges showed that the 6- to 11-meter range was the most productive in number, with a mean of 1,036 organisms per square meter (table 14). This large production was composed principally of Oligochaetes (*Tubifex*), *Gammarus*, and *Hyallela*. *Gammarus* extended in moderate abundance to 23 meters, but the largest concentration was in the 1- to 5-meter range. Tendipedids were most abundant in the 6- to 11-meter range, but were numerous down to 92 meters. Sphaeriids were common at 1- to 5-meters. The highest numbers of *Gammarus* were found around aquatic plant beds of *Potamogeton pusillus* and *Lemna trisulca*, but fish predation (to be discussed later) undoubtedly influenced these numbers.

TABLE 14.—*Mean number of bottom organisms collected per square meter at various depth ranges in Yellowstone Lake, June 15 to September 18, 1957, and July 15 to August 30, 1958*

[N=number collected; F=frequency of occurrence]

Organism	Collected at depths of—											
	0–5 meters (16 samples)		6–11 meters (32 samples)		12–17 meters (30 samples)		18–23 meters (11 samples)		24–58 meters (21 samples)		61–92 meters (5 samples)	
	N	F	N	F	N	F	N	F	N	F	N	F
Oligocheata	5	4	366	14	23	15	36	7	42	14	39	3
Hirudinea	20	6	70	21	7	6	0	0	13	3	4	1
Sphaeriidae	22	7	4	3	4	5	4	1	4	2	0	0
Tendipedidae	34	8	83	20	295	25	245	10	497	21	280	3
Gammarus lacustris	537	15	295	28	158	21	178	6	54	9	0	0
Hyallela azteca	153	8	216	14	84	6	3	1	0	0	0	0
Trichoptera	11	4	2	2	0	0	0	0	0	0	0	0
Ephemeroptera	2	2	0	0	0	0	0	0	0	0	0	0
Hydracarina	3	1	0	0	0	0	0	0	0	0	0	0
Gastropoda	1	1	0	0	1	2	0	0	0	0	0	0
Total	788	--------	1,036	--------	572	--------	466	--------	610	--------	323	--------

The number of organisms per square meter is low when compared with those found in similar studies. Reimers, Maciolek, and Pister (1955) reported more organisms per unit area for their lakes in the Sierras, although only Lake Mildred contained many amphipods. The number per square meter also was lower down to 60 meters than Rawson (1953) reported for Great Slave Lake. The latter author also compared the percentage composition of the dominant kinds of bottom organisms from several large North American lakes; the Yellowstone Lake bottom fauna was similar, except that *Gammarus* replaced *Pontoporeia* as the dominant amphipod. Calhoun (1944a) found a higher standing crop in Blue Lake, Calif., than in Yellowstone, but found a rapid drop in production below 20 meters.

The number of bottom organisms in different areas of Yellowstone Lake was compared from 2 to 15 meters (table 15). The largest numbers were collected off Lake Area in the northern area of the lake. The lowest number was off Clear Creek, which is an exposed wave-swept shoreline where organic matter does not accumulate. A lower number than would be expected was present in the South Arm and near Frank Island. Most of the differences between these latter two stations and lake were in numbers of *Gammarus*, which is an important food for cutthroat trout. The low production in Southeast Arm was possibly due to a large accumulation of fine silt and to the shifting bottom.

TABLE 15.—*Number of bottom organisms per square meter at six stations in Yellowstone Lake in 1958*

[Samples ranged in depth from 2 to 15 meters at about 3-meter intervals]

Organism	West Thumb off Solution Creek, August 5; bottom sand with little silt (6 samples)	Southeast Arm off Trail Creek, July 31; bottom fine silt with little sand (6 samples)	South Arm near Peale Island, August 12; bottom sand, gravel, and silt (6 samples)	Off Clear Creek, July 25; bottom sand, gravel, little silt (4 samples)	North side of Frank Island, August 7; bottom sand, gravel, and silt (5 samples)	Lake Area, July 28 and August 15; bottom sand, gravel, and silt (6 samples)
Oligochaeta	0	2	20	0	4	24
Hirudinea	0	0	32	11	32	48
Hyallela azteca	203	34	54	30	176	602
Gammarus lacustris	317	99	206	99	435	919
Hydracarina	7	0	0	0	0	11
Ephemeroptera	4	0	0	0	0	3
Trichoptera	11	2	0	0	0	0
Tendipedidae	39	66	63	27	54	108
Gastropoda	0	2	0	0	0	0
Sphaeriidae	4	11	18	0	15	18
Total	585	216	393	169	716	1,733
Mean number per sample	99.5	36.0	65.5	42.3	143.2	288.8

VERTICAL DISTRIBUTION OF CUTTHROAT TROUT

During the open-water season, cutthroat trout in Yellowstone Lake rarely venture below 20-meter depths. During 1957 and 1958 more than 99 percent of the cutthroat trout were captured in the upper 25 meters of water, in 92 gill-net sets (table 16). Of the total, 63 percent were collected from the surface to 15 meters, and no trout were captured below 30 meters. This type of vertical distribution of cutthroat trout has been documented by McConnell, Clark, and Sigler (1957) for Bear Lake in Utah and Idaho, although the cutthroat population was small.

Our data do not conclusively show great variations in vertical distribution among different size groups, but small trout (155–200 mm., predominently age group II) frequented water below 20 meters more than did larger trout.

TABLE 16.—*Number of cutthroat by size groups captured by 92 overnight experimental gill-net sets at different depth ranges in Yellowstone Lake during July and August of 1957 and 1958*

Depth	Number of sets	Number in size group range—								Total
		Less than 100 mm.	105–150 mm.	155–200 mm.	205–250 mm.	255–300 mm.	305–350 mm.	355–400 mm.	Above 400 mm.	
0–5 meters	11	0	0	25	30	45	55	60	4	219
6–9 meters	11	0	0	51	30	36	57	39	1	214
10–15 meters	22	0	0	40	16	21	38	43	2	160
17–20 meters	11	0	0	6	3	3	25	28	0	65
21–25 meters	22	1	0	23	3	0	10	9	0	46
26–30 meters	8	0	0	1	2	1	1	1	0	6
31–46 meters	7	0	0	0	0	0	0	0	0	0
Total	92	1	0	146	84	106	186	180	7	710

FOOD OF CUTTHROAT TROUT

The most abundant organisms found in the stomachs of cutthroat trout in Yellowstone Lake were *Daphnia shoedleri*, *Gammarus lacustris*, and various species of Tendipedidae (table 17). *Diaptomus shoshone*, Trichoptera, Plecoptera, and Ephemeroptera occurred in fewer stomachs. The seasonal changes in feeding habits showed that *Gammarus* was most important in mid-July, but declined in importance in August. The number of *Daphnia* increased from July to October and is believed due to the swarming habit during the latter part of the summer. Tendipedidae pupae and adults were common in stomachs during July or when the major emergences occurred. Tendipedidae larvae were not utilized heavily. *Diaptomus* occurred in few stomachs in July, but assumed greater importance later in the season. Only three stomachs out of 409 contained fish, and these were cutthroat trout.

Feeding habits of trout by size groups showed that both *Gammarus lacustris* and *Daphnia schoedleri* were the most commonly utilized foods of all sizes of trout (table 18). There was a gradual transition in the range of 275 to 325 mm. from a zooplankton-bottom fauna diet to a predominantly bottom fauna diet. *Diaptomus* were rarely utilized above 250 mm., and *Ephemerella* nymphs and Trichoptera were most important among large fish. From our knowledge of the distribution of the food organisms in the lake, it appears that small trout feed both in the limnetic and littoral zones while large trout feed almost entirely in the littoral zone. *Daphnia schoedleri*, the only zooplankter found in many stomachs of large trout, is an inshore swarming species.

Differences in feeding habits in two sections of Yellowstone Lake were demonstrated from the stomach contents of 344 cutthroat trout caught at Fishing Bridge and West Thumb docks (table 19). Stomachs from fish caught at Fishing Bridge contained many stream organisms and some trout eggs. Trout from West Thumb utilized terrestrial organisms more so than trout from Fishing Bridge. Many stomachs from Fishing Bridge contained both *Diaptomus* or *Daphnia* and stream bottom insects, which indicates that cutthroat trout can rapidly change their feeding habits to utilize a different type of food. The large number of terrestrial insects from West Thumb included the wind-blown bark beetles, wasps, and others, which were carried into the water by the predominant south and southwesterly winds from the lodgepole-pine forests.

Calhoun (1944b) found that cutthroat trout (*Salmo clarkii henshawi*) utilized *Tendipes* larvae more commonly than

TABLE 17.—*Stomach contents of 409 cutthroat trout captured by gill nets, by time of capture in 1958*

[All sizes combined. P=percent of stomachs containing organisms; M=mean number, to nearest 1.0, of food organisms per stomach. Collected from South Arm, Lake Area, West Thumb, and Southeast Arm]

Food organism	July 15–31 (238 fish)		August 1–15 (100 fish)		August 16–31 (43 fish)		October 15 (28 fish)	
	P	M	P	M	P	M	P	M
Daphnia	19.7	209	24.0	295	37.2	72	82.1	976
Diaptomus	2.1	223	3.0	541	7.0	158	14.2	93
Gammarus lacustris	56.7	25	41.0	27	30.2	9	17.8	105
Hyallela azteca	1.3	5	1.0	15	2.3	65	0	0
Hydracarina	2.5	5	0	0	0	0	0	0
Plecoptera	1.3	1	2.0	2	4.7	1	0	0
Ephemerella	10.1	13	3.0	3	0	0	0	0
Trichoptera	6.3	13	8.0	4	0	0	0	0
Tendipes larva	8.8	19	4.0	2	0	0	0	0
Tendipes pupa	23.5	20	10.0	11	4.7	2	0	0
Tendipes adult	20.6	28	8.0	4	2.3	1	0	0
Prodiamesa	0.4	1	0	0	0	0	0	0
Procladius	1.3	4	0	0	0	0	0	0
Hymenoptera and Coleoptera	1.7	8	1.0	2	0	0	7.1	13
Gastropoda	2.1	5	0	0	0	0	0	0
Pisidium	0.8	3	0	0	0	0	0	0
Trout eggs	0.4	4	0	0	0	0	0	0
Trout	0.4	1	0	0	2.3	4	3.6	1
Number of stomachs empty	30		16		11		0	

TABLE 18.—*Stomach contents of 298 cutthroat trout captured by gill nets during July and August 1958, by length groups*

[P=percent of stomachs containing organisms; M=mean number of organisms per stomach, to nearest 1.0]

Food organisms	Trout size range													
	150–200 (41 fish)		205–250 (66 fish)		255–275 (30 fish)		280–300 (19 fish)		305–325 (37 fish)		330–350 (45 fish)		355–400 (60 fish)	
	P	M	P	M	P	M	P	M	P	M	P	M	P	M
Daphnia	43.9	381	42.4	477	33.3	349	52.6	749	24.3	343	24.4	267	25.0	189
Diaptomus	12.2	215	7.6	273	0	0	0	0	0	0	2.2	200	1.7	80
Gammarus	36.6	13	39.4	24	36.7	11	57.9	32	40.5	24	44.4	26	51.7	30
Hyallela	7.3	6	0	0	0	0	0	0	2.7	15	2.2	65	0	0
Hydracarina	0	0	3.0	10	0	0	5.3	1	5.4	4	0	0	3.3	3
Plecoptera	2.4	1	1.5	1	0	0	0	0	2.7	1	0	0	0	0
Ephemerella	9.8	2	10.6	2	6.6	16	5.3	2	2.7	1	4.4	33	3.3	48
Trichoptera	0	0	4.5	2	1	75	5.3	1	13.5	7	4.4	5	1.7	24
Tendipes larva	4.9	3	4.5	15	3.3	49	5.3	3	0	0	2.2	3	6.7	25
Tendipes pupa	17.1	14	9.0	15	16.6	26	21.0	5	8.1	15	24.4	17	20.0	32
Tendipes adult	7.3	24	10.6	17	20.0	42	0	0	5.4	29.	15.6	5	8.3	54
Prodiamesa	2.4	125	0	0	0	0	0	0	0	0	0	0	0	0
Procladius	0	0	0	0	0	0	5.3	3	0	0	2.2	8	1.7	2
Hymenoptera and Coleoptera	0	0	1.5	2	3.3	20	0	0	5.4	5	2.2	2	1.7	2
Gastropoda	0	0	1.5	7	0	0	0	0	0	0	2.2	7	1.7	2
Pisidium	0	0	0	0	0	0	0	0	0	0	0	0	3.3	3
Trout	0	0	0	0	0	0	0	0	2.7	1	0	0	1.7	1
Number of stomachs empty	8		5		4		2		8		6		17	

pupae in Blue Lake, due to a scarcity of other food organisms. He also found that plankton crustacea were used more commonly than *Gammarus* and immature chironomids in a more productive lake. Some workers have mentioned the piscivorous nature of cutthroat trout (Echo, 1956; McConnell, et al., 1957) when large populations of forage fishes are present. Irving (1956) found mostly *Gammarus* and *Tendipes*, but few fish, in cutthroat trout from Henrys Lake, in spite of the large number of forage species available. In general, cutthroat trout food studies have shown a preference for macrozooplankters and bottom organisms over fish, except where unusually large cutthroat occur.

TABLE 19.—*Stomach contents of 344 cutthroat trout caught by angling in the vicinity of Fishing Bridge and West Thumb docks from June 5 to August 15 in 1957 and 1958*

[P = percent of stomachs containing organisms; M = mean number of organisms per stomach, to nearest 1.0]

Food organism	Fishing Bridge (169 fish)		West Thumb (175 fish)	
	P	M	P	M
Gordius	2.4	4	0	0
Daphnia	11.2	171	8.6	128
Diaptomus	10.7	1,213	16.6	714
Gammarus lacustris	18.9	12	33.7	21
Hyallela azteca	0.6	145	0	0
Plecoptera	8.9	7	2.3	3
Ephemeroptera	14.2	14	12.0	34
Odonata	0	0	0.6	1
Hemiptera	0	0	1.1	3
Trichoptera	10.7	6	3.4	8
Hymenoptera and Coleoptera	4.1	8	16.0	10
Tendipes larva	6.5	7	4.6	6
Tendipes pupa	20.7	30	37.7	22
Tendipes adult	5.9	10	22.9	31
Procladius	1.8	16	0	0
Pisidium	1.8	2	0	0
Cutthroat trout and eggs	11.2	8	0	0
Number of stomachs empty	38		15	

TROUT DENSITY AND LIMNOLOGY

Angling pressure on Yellowstone Lake is concentrated in the northern area and, to a lesser extent, in West Thumb, owing to the lack of access in the southern sections. The total catch by area from 1950 to 1959 showed that an average of 66.7 percent of the fish were removed from the northern area, an average of 24.8 percent from West Thumb, and an average of 8.5 percent from the southern area (table 20). The northern area occupies 25.4 percent of the lake area, West Thumb 19.1 percent, and the southern area 55.5 percent.

Three possible effects of this unequal harvesting of trout in the northern area were (1) fewer trout in relation to food abundance, (2) large numbers of bottom organisms owing to lack of predation, and (3) movements of trout into the area.

First, gill-netting in different sections of the lake showed fewer trout off Lake Area than would be expected from the habitat and the large amount of food (table 21). All gill-netting was carried out after the major spawning runs had stopped. The largest number were caught in the South Arm and around Frank Island. The scarcity of trout off Clear Creek was due to the exposed wind-swept nature of the shoreline. The scarcity of trout in Southeast Arm is believed to be due to the low food production and the fact that gill-netting was on the delta of the Yellowstone River, which cutthroat do not frequent because of the shifting bottom.

TABLE 20.—*Percentage of trout caught in each of three areas of Yellowstone Lake, by years, 1950 to 1959*

Year	Percent caught in—			Number caught
	Northern area	West Thumb area	Southern area	
1950	69.4	24.6	6.0	200,015
1951	69.3	24.3	6.4	208,255
1952	68.7	23.4	7.9	245,277
1953	66.6	23.7	9.6	195,873
1954	69.1	22.0	8.9	215,933
1955	66.9	25.1	8.0	286,056
1956	64.0	25.6	10.4	290,221
1957	63.4	26.9	9.7	301,155
1958	64.7	25.3	10.0	349,027
1959	64.6	27.1	8.3	393,467
Mean	66.7	24.8	8.5	------------

Table 21.—*Number of trout of more than 200 millimeters, total length, captured in depths of 0 to 20 meters in six areas of Yellowstone Lake by 38 overnight experimental gill-net sets in 1958*

Sampling area	Date of set	Number of sets	Number of trout caught	Mean catch per set
Lake	July 25–Aug. 18.	6	66	11.0
Middle South Arm	Aug. 13	3	47	15.6
Peale Island	July 15–24	14	219	15.6
Southeast Arm	July 31	3	29	9.6
Clear Creek	Aug. 19	3	20	6.6
Frank Island and Plover Point.	Aug. 8–13	9	125	13.9

Second, bottom organisms in the northern area were more numerous than would normally be expected from the bottom types or abundance of aquatic plants (table 15). The number of organisms above 15 meters at Lake Area averaged 288.8, whereas the nearest recorded number in other sections of the lake was 143.2 at Frank Island. The greater abundance of bottom organisms in the northern area was due to more *Gammarus* and *Hyallela*, the principal foods of the cutthroat trout. The most plausible reason for the large number of bottom organisms at Lake Area is the lack of trout predation on bottom organisms due to rapid recruitment by angling. Both Ball and Hayne (1952) and Reimers (1958) demonstrated that fish populations can severely deplete bottom fauna populations. Wohlschlag (1950) found that bluegills fed more heavily in areas of heavy chironomid production than in areas of low production.

Third, trout apparently moved into the northern area. This movement was believed to be for feeding or to take advantage of the high standing crop of bottom food, since most movement occurred after the spawning period. From 1949 to 1955, 18,836 trout were tagged in five spawning streams, and the returned tags that included sufficient information on catch location were used to compare the general movements of postspawning trout from five spawning streams (table 22), (Ball and Cope 1961). The data on fish movement can be summarized as follows: Those fish tagged in Pelican Creek were usually recovered in the northern area and around Fishing Bridge. Clear Creek fish also were captured in the northern area, particularly in Bridge Bay. Fish tagged in Chipmunk and Grouse Creeks, at the lower end of South Arm, were most commonly captured in the southern area or in West Thumb, although 25 percent were captured in the northern area. There was a movement of postspawners from Chipmunk and Grouse into West Thumb and, to a lesser extent, into the northern area in August, and many were captured in these locations during the year following tagging. From the movements of postspawners, the only evidence of a long-distance movement from the spawning stream to the area of capture was the large number of fish tagged in South Arm that moved into West Thumb and into the northern area. A lesser movement was apparent from those fish tagged in Arnica Creek and recovered on the west shore of the northern area of the lake. In general the postspawners recovered the second year after tagging dispersed more than those caught the first year.

Table 22.—*Movements of cutthroat trout after spawning, as determined from fish tagged in five spawning streams and recaptured by angling, 1949–55*

Spawning stream	Number of fish tagged	Number of tag returns	Percent of all returns captured in—		
			Northern area [1]	Southern area	West Thumb area
Arnica Creek	4,948	271	26.2	4.4	69.3
Pelican Creek	6,043	422	88.9	4.5	6.6
Clear Creek	1,100	54	70.3	12.9	16.7
Chipmunk and Grouse Creeks	6,745	160	25.0	41.9	33.1
Total	18,836	907			

[1] Includes 18 returns from Yellowstone River.

Heavy fishing pressure undoubtedly influenced the number of tag returns from the northern area. The estimated annual fishing pressures, in hours per acre, from 1950 to 1954 were northern 10.5, West Thumb 4.9, and southern 0.6. The fishing mortality rates (percent) for postspawning trout from five spawning streams were as follows: Pelican, 12.2; Arnica, 9.3; Chipmunk, 5.2; Grouse, 3.7; and Clear, 9.4 (Ball and Cope 1961). These fishing mortality rates show that fish from Pelican, Arnica, and Clear Creeks are subjected to a greater fishing pressure than fish from Chipmunk and Grouse, but the ratios are not so different as the fishing pressure per acre for the three areas of the lake; thus, there must have been a greater movement of trout into the northern area or to West Thumb than away from it.

From the location of tag recoveries, there appeared to be a tendency for postspawning trout to follow the shoreline of greater standing crops of food organisms from their spawning streams to their point of capture, although such was not always true. It was particularly true, however, for fish from Chipmunk, Grouse, and Arnica Creek. These data support the hypothesis that feeding may be an important factor in causing trout to move out after spawning.

There was a greater concentration of planktonic entomostraca in the northeast part of the lake due to surface currents, but the food of postspawning trout is principally bottom organisms, and a large standing crop of bottom foods would be more important than plankton in causing any fish to migrate.

BIOLOGY OF YELLOWSTONE LAKE

Yellowstone Lake has many characteristics of the large oligotrophic lakes of Canada or of the northern Great Lakes, but its bottom fauna and fish compositions are notably different. The other lakes have *Pontoporeia* or *Mysis* as the dominant bottom fish-food organism, and all have large populations of coregonids. Undoubtedly, factors which have prevented introduction of these organisms into Yellowstone Lake include the impassable falls 15 miles below the lake outlet and the altitude.

An interesting comparison between the mean depths of the large lakes of Canada and the northern Great Lakes and the annual commercial fish production (lbs. per acre) has been prepared by Rawson (1955). He constructed a curve and found that fish production decreased with increasing depth on a relatively smooth curve. A comparison of the mean depth of Yellowstone Lake (139 ft.) and its mean sport-fish production from 1950 to 1959 (2.15 lbs. per acre) shows that it fits into this curve closely between Lake Nipigon (1.07 lbs. per acre, and mean depth 180 ft.) and Lake Winnipeg (2.66 lbs. per acre, and mean depth 43 ft.). It is surprising that the sport-fishery production of Yellowstone Lake with only one fish species fits so well into this curve when the commercial fish production of these lakes involves many fish species and several fish predator-and-prey relations.

The limnological classification of Yellowstone Lake according to Lundbeck (1934) would be primary oligotrophic, since it is poor in nutrients and has low temperatures. The volcanic nature of the drainage, with deficiencies in calcium and other minerals, probably has as great an influence in limiting production as has morphometry. Turbidity and too great a water exchange are not limiting factors in Yellowstone as mentioned for Port John Lake, British Columbia (Robertson, 1954), which has a similar water chemistry but a high rate of water replacement. The low number of plankters per liter in

Yellowstone Lake is partly offset by the depth of its trophogenic zone. Ohle (1956) stated that deep lakes may have a lower production density, but this deficiency is compensated for by a greater production depth than shallow lakes. The great production depth of Yellowstone Lake is shown from the depth distribution of aquatic plants, bottom fauna, and plankton. Secchi disk records, which reflect plankton density, showed high readings.

The development and use of Yellowstone Lake is controlled by the National Park Service. This control has prevented artificial eutrophication as has occurred in several large oligotrophic lakes (Edmundson et al., 1956). The data collected by Forbes (1893) in 1890 were not quantitative, but a comparison of his observations with the data in this report indicates that the dominant species composition of zooplankters, phytoplankters, and bottom organisms did not change from 1890 to 1957.

Few large lakes have as few fish species as Yellowstone, but it appears that the cutthroat are utilizing the available animal food rather efficiently. The depth range of the trout is closely related to the depth distribution of both the predominant bottom foods and planktonic foods. The kinds of food utilized by different sizes of trout overlap to a great degree, although there is a gradual transition from a bottom fauna–zooplankton diet to a predominantly bottom-fauna diet in the range of 275 to 325 mm. (table 18). This change in feeding habits occurs in the same length range when trout first become available to the sport fishery in numbers. A length frequency curve of the catch for 1959 shows that the slope begins to increase around 275 mm., but that the greatest increase takes place above 315 mm. (fig. 8). The reason the catch does not include more small trout (below 12 inches) is not that they are caught and returned to the lake, since many anglers will keep trout around 10 inches. These trout are difficult to catch[1] and are dispersed more in the lake owing to their feeding habits. Small trout feed both in the limnetic and littoral zone, while large trout feed in the littoral zone. The greater dispersal of small trout makes them less vulnerable to angling. Another probable effect of this great dispersal is the low rate of predation on small trout by large trout as shown by food studies. This fact also accounts for the high survival rate of 36 percent which Ball and Cope (1961) suggest exists from the time that immature fish leave the parent stream until they return as spawners.

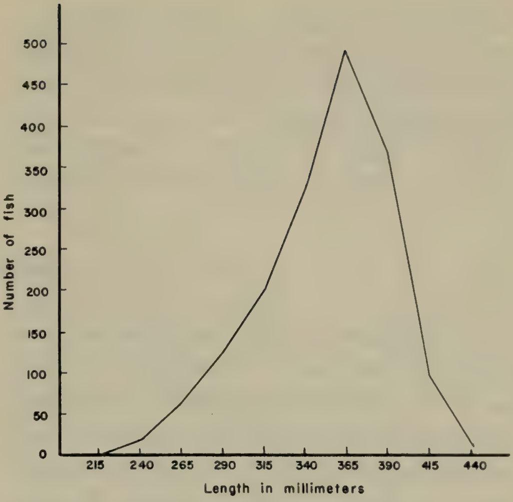

FIGURE 8.—Length frequency of anglers' catch from Yellowstone Lake in 1959 from sample of 1,690 fish. Points represent medians of 25-mm. groups.

The only competitive species appears to be the longnose sucker, since recent studies (unpublished) have shown that suckers feed primarily on the same bottom organisms as the cutthroat trout. Past data on the sucker population in Yellowstone Lake are lacking, although records from the Yellowstone Fish Cultural Station indicate that the sucker was introduced in 1923 or 1924. Numbers of suckers recorded in the spawning runs in Pelican Creek showed a general increase up to 1950 and a leveling off since that year. The competition with the cutthroat trout appears to be increasing, although there has been no clear evidence of detrimental influence on the trout population.

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[1] Madsen in an unpublished report (Age and growth of the cutthroat trout (*Salmo lewisi*) in Yellowstone Lake, Wyoming, dated May 15, 1940) attempted to determine why anglers rarely caught small trout and found that small trout would rarely take any lure, even when schools were located.

SUMMARY

Limnological studies on Yellowstone Lake, Yellowstone National Park, were conducted from 1954 to 1959. Geological evidence indicates that the original basin was formed from a subsidence or readjustment of the laval flows in the Eocene. Hot springs still exist in the drainage basin and in the lake. Annual water-level fluctuation is less than 1.8 meters. The lake lies at an altitude of 2,358 meters and has the following dimensions: Surface area, 35,391 hectares; mean depth, 42 meters; basin capacity, 14 x 10^9 cubic meters; maximum depth, 95 meters; and shoreline development, 3.04. A contour map is included.

Water-current studies showed a large surface-water movement from the south and southwest to the north and northeast parts of the lake. Compensatory subsurface currents were found by bottom-current studies. Water-temperature data showed various stages of stratification from late May and early June when the ice left until late July when complete stratification was evident. Differences in water stratification between West Thumb and the northeast part of the lake were related to surface-water movement. High water levels in certain years were associated with deep epilimnion layers.

Water composition showed HCO_3 to be the major anion and Na to be the major cation. Conductivities were highest in West Thumb and lowest in Southeast Arm. Upwelling is believed to have caused the high readings in West Thumb. Protected stations had lower conductivities than exposed stations, and the causative factor was believed to be the thinner epilimnion strata in the protected stations which caused a more rapid utilization of nutrients by plankton. Oxygen concentrations were usually above or near saturation, although deficits developed in the hypolimnion during 1957 and 1959. Free carbon dioxide was present in all samples and concentrations above 10 p.p.m. were found below the metalimnion.

Bottom types included boulders, rubble, black obsidian sand, and fine clays with organic matter. Amount of organic matter in the soft sediments increased with depth, but lake areas with strong currents had low amounts of organic matter. Greatest concentrations of soil nutrients were in deep water. A list of aquatic plants and their depth ranges is presented. The depth limit of all higher aquatic plants was 18 meters.

The diatoms *Asterionella*, *Melosira*, and *Stephanodisus* were abundant. A dense pulse of *Anabaena* was observed in August. Principal zooplankters were *Conochilus unicornis*, *Diaptomus shoshone*, and *Daphnia shoedleri;* largest concentrations ranged from 5 to 18 meters. Several large swarms of *Daphnia shoedelri*, an inshore swarming species, were observed in 1959. Benthos consisted principally of *Gammarus lacustris*, *Hyallela azteca*, oligochaetes, and tendipedids. Ex-

cept for tendipedids, which were present at all depths, bottom organisms were rare below 23 meters.

Cutthroat trout were concentrated in the upper 15 meters of water and were rare below 25 meters. Most abundant food organisms in trout stomachs in the summer season were *Gammarus lacustris*, *Daphnia shoedleri*, and tendipedids. *Gammarus* were important in June and July, and *Daphnia* in August and September. Small trout utilized both bottom organisms and zooplankton, while large trout fed almost entirely on bottom organisms.

Angling pressure and catch were concentrated in the northern area and three effects of this unequal harvesting were (1) fewer trout in relation to food abundance, (2) large numbers of bottom organisms owing to lack of predation, and (3) movements of trout into the area.

Yellowstone Lake is oligotrophic and has a deep productive depth. The dominant species composition of plankton and bottom organisms has not changed from 1890 to the present. Low availability of small trout to anglers is caused by feeding habits and great dispersal; this serves to prevent overexploitation of small trout.

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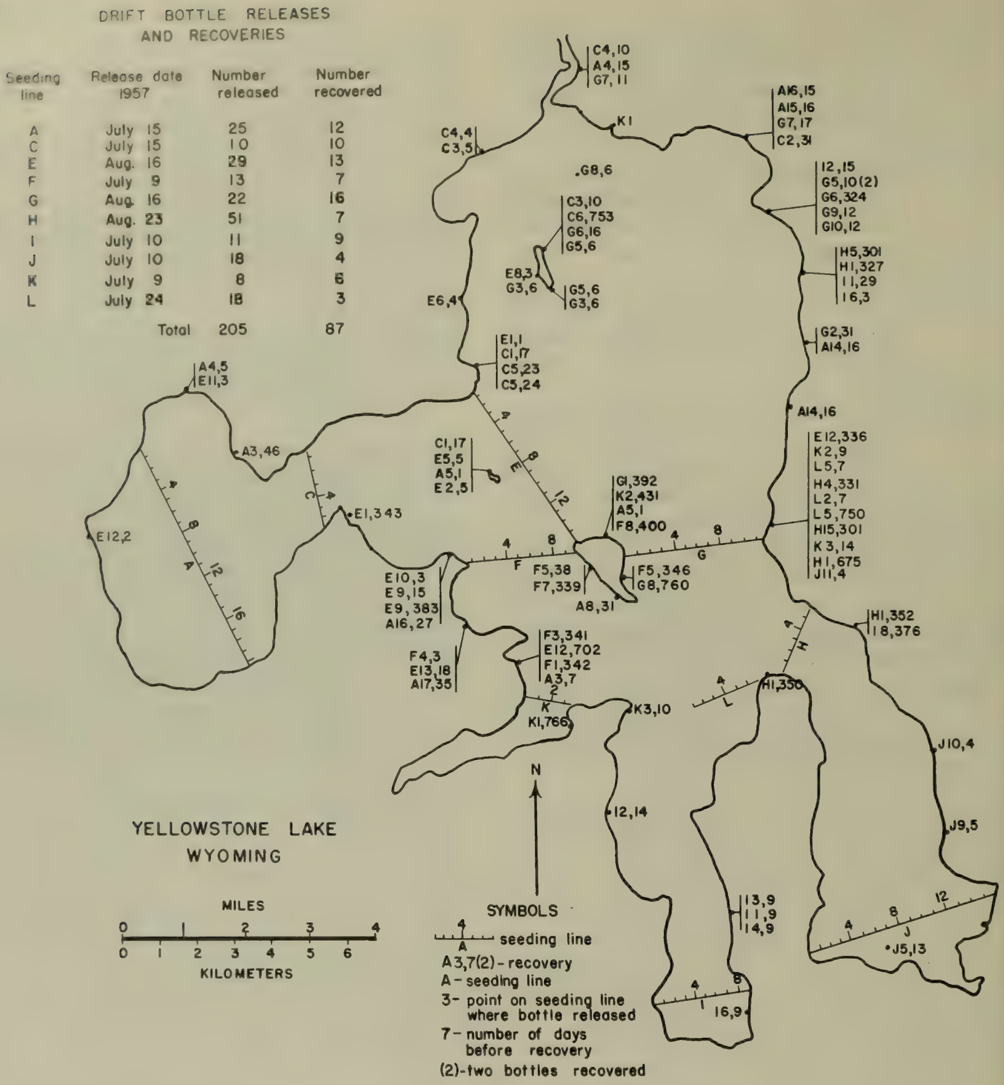


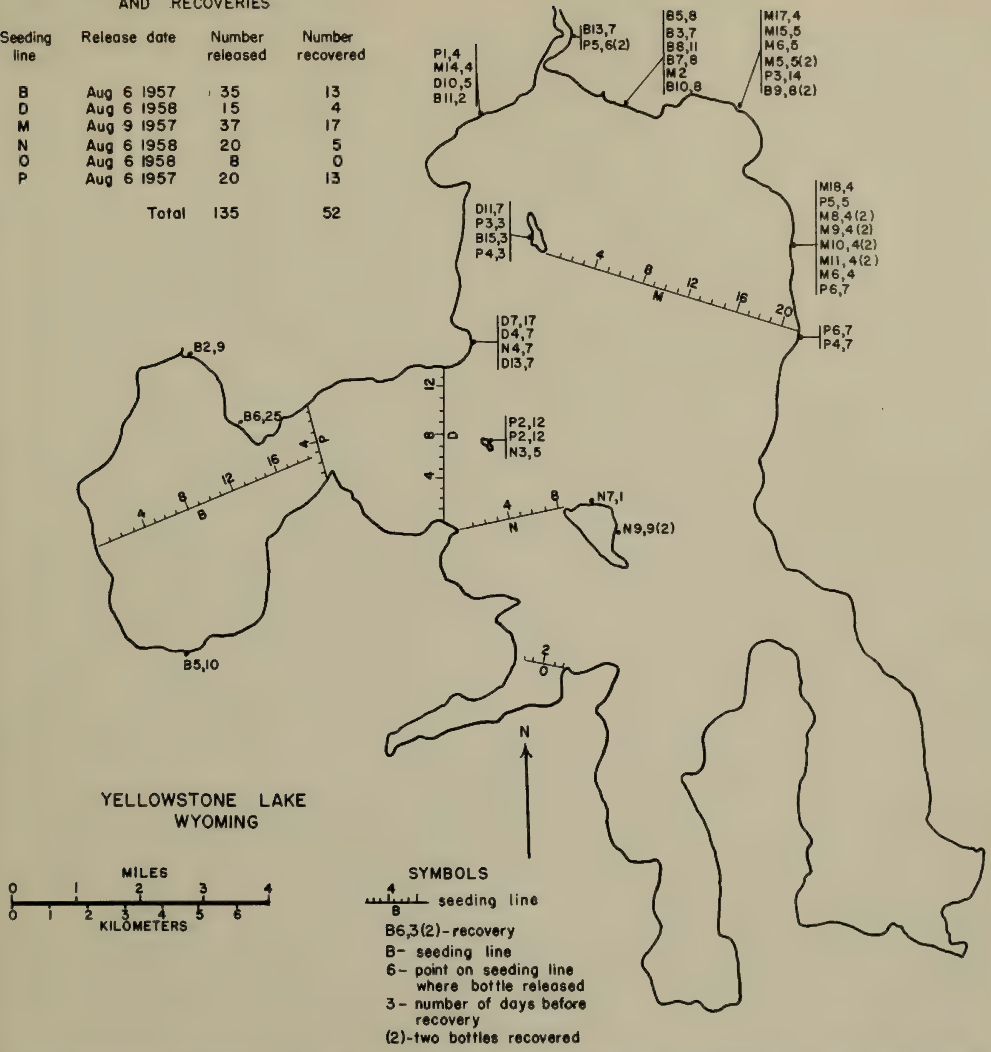
DRIFT BOTTLE RELEASES AND RECOVERIES

Seeding line	Release date 1957	Number released	Number recovered
A	July 15	25	12
C	July 15	10	10
E	Aug. 16	29	13
F	July 9	13	7
G	Aug. 16	22	16
H	Aug. 23	51	7
I	July 10	11	9
J	July 10	18	4
K	July 9	8	6
L	July 24	18	3
	Total	205	87

Appendix A.—Points of release and recovery of drift bottles in Yellowstone Lake, Wyoming.

DRIFT BOTTLE RELEASES
AND RECOVERIES

Seeding line	Release date	Number released	Number recovered
B	Aug 6 1957	35	13
D	Aug 6 1958	15	4
M	Aug 9 1957	37	17
N	Aug 6 1958	20	5
O	Aug 6 1958	8	0
P	Aug 6 1957	20	13
	Total	135	52



APPENDIX B.—Points of release and recovery of drift bottles in Yellowstone Lake, Wyoming.

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CHEMICAL COMPOSITION OF BLOOD OF SMALLMOUTH BASS

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 57

GOVERNMENT
PUBLICATIONS
FEB 1 3 1962

RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

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57. Chemical Composition of Blood of Smallmouth Bass, by Eddie Wayne Shell. 1961. 36 p.
58. Chronic Effects of Endrin on Bluntnose Minnows and Guppies, by Donald Irvin Mount. In press.

CHEMICAL COMPOSITION OF BLOOD OF SMALLMOUTH BASS

Eddie Wayne Shell
Fishery Research Biologist

RESEARCH REPORT 57

UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, *Secretary*
Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*

FISH AND WILDLIFE SERVICE
Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE
Daniel H. Janzen, *Director*

March 3, 1849

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ABSTRACT

Concentrations of 16 organic and inorganic components of the blood and serum of the smallmouth bass (*Micropterus dolomieui*) were measured every 3 to 4 weeks from June 24 to November 18, 1958. Components measured included proteins, nonprotein nitrogen fractions, phosphorus fractions, electrolytes, and cholesterol in serum; and glucose, creatinine, and iron in blood.

Concentrations of 13 of the 16 components displayed systematic rather than random variation and appeared to be cyclic when plotted against time. Levels of several of the components decreased in successive samples during the early part of the experimental period only to reverse this trend and increase throughout the remainder of the period. Other components displayed inverse behavior to this scheme.

Three periods of different catabolic-anabolic balance have been proposed as being responsible for the cyclic nature of the concentration curves. Available evidence suggests that endocrine relations are responsible for changes noted in blood composition. A scheme of hormone-hormone antagonism is proposed, which would account for the observed results.

CHEMICAL COMPOSITION OF BLOOD OF SMALLMOUTH BASS

By Eddie Wayne Shell, *Fishery Research Biologist*

Cornell University, Ithaca, N.Y.

No problem is more fundamental to understanding total metabolism than is the relation of composition to function in the blood of animals. No single component or part of an animal is more closely associated with total metabolism than is blood. Every organ, every tissue, every cell communicates with every other organ, tissue, and cell by this fluid "party line." The composition of the blood at any one time reflects the state of function of every organ, tissue, and cell at that time. If the composition of blood and the relation of composition to function are known, then it would be possible to determine the state of function at any time by measuring composition. Such information could also be used to predict trends in function.

Clinical diagnosis used so effectively by modern medicine is dependent on information on the composition of blood and the relation of its composition to function. Many specific diseases, whether caused by bacteria or metabolic dysfunction, are characterized by specific changes in the composition of the blood of the animal. Clinical diagnosis depends on the comparison of the blood composition of the animal with a suspected disease with the blood composition of healthy animals of the same species. Obviously, information as to the levels of the various components in the blood of healthy animals and of animals known to have a specific disease must be available.

Information on the blood composition of healthy fish or fish with specific diseases is very scarce. With such information kidney disease, pancreatic necrosis, or other diseases in populations of hatchery fish might be diagnosed before the epidemic stage is reached. Vitamin deficiencies or other nutritional diseases could be detected much earlier than is possible when decreasing growth rates or increasing mortality rates are used as a basis for diagnosis. This project was initiated to provide information on the composition of the blood of normal or apparently healthy fish.

Specifically, this project was initiated to determine the levels of 16 noncellular components in the blood of the smallmouth bass (*Micropterus dolomieui* Lacépède). These components were to be measured periodically over approximately a 6-month period to determine average values, or if the levels changed significantly in this period, determine the extent of change and attempt to relate change in composition to function in the cyclic life patterns of this fish.

This investigation was conducted during the period June through November 1958, by the author while a student at

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Presented to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author is presently at Auburn University, Auburn, Alabama.

Research Report 57. Approved for publication, January 9, 1961.

Cornell University. The laboratory work was conducted at the Cornell University experimental fish hatchery. Smallmouth bass used in the investigation were obtained from a creek adjacent to the campus.

Dr. Arthur M. Phillips, Jr., Associate Professor of Fishery Biology, Cornell University, proposed this study and made many helpful suggestions throughout its course. Dr. Harry W. Seeley, Jr., Professor of Bacteriology, and Dr. Walter L. Nelson, Professor of Biochemistry, reviewed the manuscript.

COLLECTION AND TREATMENT OF SAMPLES

EXPERIMENTAL ANIMALS

Difficulties in maintaining experimental animals under laboratory conditions made the use of a natural population almost mandatory. The bass used in these experiments were taken from Fall Creek, a small creek which receives overflow waters from Lake Como, a few miles southeast of Dresserville, Cayuga County, New York. From Lake Como the creek flows southeastward until it enters Tompkins County, thence to Ithaca, N.Y., where it empties into Cayuga Lake. Fish used in these experiments were taken from a section limited on the north by Malloryville, N.Y., and on the south by a dam at the north end of Beebe Lake on the campus of Cornell University. This section is generally coincident with the area of acceptable bass habitat in Fall Creek.

The fish are more or less evenly distributed from May until September throughout the section described. During the latter part of September or early October, when the water temperature begins falling, the fish tend to move into a few restricted areas of this section where they spend the winter months in a quiescent state. These areas are characterized by bottoms strewn with large flat rocks. This migration or movement to wintering quarters begins when water temperatures are around 50° to 55° F., in late September or early October, and is completed by the end of October when temperatures range from 45° to 50° F. The quiescent state begins when the water temperatures fall below approximately 45°. Throughout the winter and until the temperature begins to rise in the spring, the fish remain underneath the rocks. Apparently they will take food during this period but do not actively seek it.

The population of fish used in these experiments is characterized by the small average size of the individuals. Fish 8 to 10 inches in length comprise the bulk of the population. Only 1 fish as large as 14 inches was taken during the experimental period.

Distribution of the fish in the stream during most of the experimental period made the use of seines, traps, and electrofishing gear impractical. All fish used in these experiments, until the beginning of the quiescent state, were taken by angling with spinning tackle. Once a fish became hooked he was forced to hand as quickly as possible to avoid tearing out the hooks. A 500-volt, variable voltage, direct-current generator with the voltage regulator set for 450 volts was used to take quiescent fish under the rocks. The shocking or electrofishing apparatus was constructed so that the positive electrode was built into a dip net and the negative electrode was attached to a sled-shaped float.

COLLECTING BLOOD SAMPLES

Apparatus

The small kit shown in figure 1 has proved satisfactory in collecting blood in

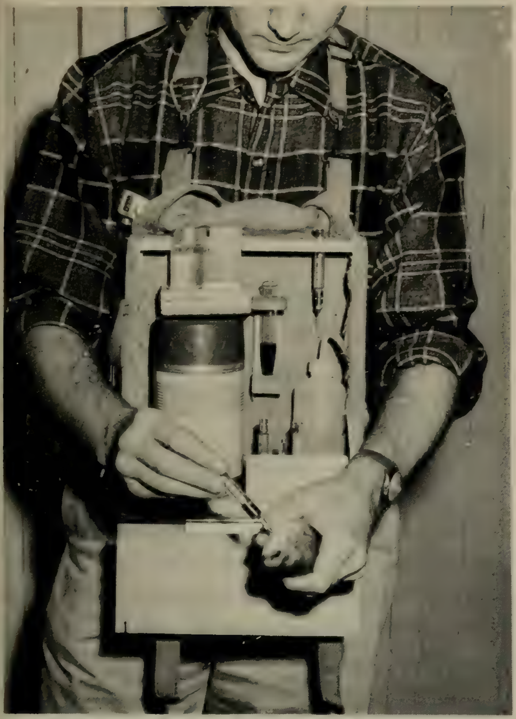

FIGURE 1.—Kit for collecting blood in the field.

the field. It consists of a wooden frame, a solid floor, a pressed-wood panel back, and a cover of canvas panels. The front is closed by a canvas panel that can be buckled to a hinged plywood panel covering the lower half of the kit. This plywood panel protects needles and syringe parts held in a draining and drying rack secured to the floor of the kit. Also attached to the floor are two slotted strips which receive the rolled edge of the bottom of a wide-mouthed vacuum bottle, 7¼ inches high and 4 inches in diameter. A small wooden rack to hold a centrifuge tube while it is being filled with blood is attached to the back of the kit. Also attached to the back is a small receptacle for holding the wash and rinse bottles of distilled water. An assembled syringe is kept in readiness in a hole in the top of the kit. A plastic bottle for reserve distilled water is held by a rubber band in the corner of the kit. In the field, the kit is carried with the aid of a canvas strap around one's neck. A large flat piece of foam rubber sewn to the strap eased support of the weight.

The vacuum bottle holds empty centrifuge tubes as well as tubes of blood in a cylindrical wire rack. The wire rack, with a piece of sponge serving as the bottom of the cylinder, holds the tubes away from the glass lining and also prevents excessive stirring of the ice and water in the vacuum bottle.

Syringes of 2-milliliter capacity with a "needle-lock" were used throughout this experiment. The syringes were large enough to hold the blood from the larger fish, and yet were small enough to be easily manipulated with one hand. Hypodermic needles ¾ inch in length and of No. 20 gage were found to be most suitable for collecting blood from the smallmouth bass. A needle of this length was needed to reach the heart in larger fish and was not unwieldy when used on smaller fish. The 20-gage needle was more practical in the prevention of hemolysis and clotting and was also much easier to keep clean.

Like other fish blood, smallmouth bass blood is highly coagulable. Small clots usually formed within about one-half minute after blood had been drawn. Clotting is of little consequence when the blood is to be used for serum; however, it was difficult to draw blood and transfer it into tubes treated with anticoagulant before clotting ruined the sample for whole blood determinations. This was overcome by preparing a 25-percent solution of potassium oxalate, drawing some of this solution into the syringe and forcing it out again. After forcing out the oxalate, air was drawn in and forcibly expelled several times. After this treatment the walls of the syringe remained moist with the anticoagulant, and blood drawn into the syringe mixed with the anticoagulant as it entered.

Procedure

Blood was collected by cardiac puncture. The needle was inserted at a 90° angle to the axis of the fish, approximately one-half inch behind the apex of the V formed by the isthmus and opercles (fig. 1). The needle point was pushed into and through the chest cavity until slight resistance was encountered. At this time suction was applied by the syringe and the needle slowly withdrawn. As the opening of the needle being withdrawn passed through a heart chamber, blood was drawn into the syringe. Best results were obtained by a slow, gentle insertion. Apparently, a needle of the size used can cause surgical shock severe enough to interrupt the beat of the heart if carelessly inserted.

The blood of a fish was collected so long as it could be easily drawn. It was usually possible to collect at least 0.5 ml. of blood, and up to 2.5 ml. from the larger fish; however, very few fish were large enough to supply 2.5 ml. of blood. The fish sampled averaged approximately 7 inches and the amount of blood collected averaged 0.75 ml. per fish. The blood was gently expelled from the syringe into a 12-ml. conical centrifuge tube. Because of the size of the needle used, blood could be forced through it into the tube without causing hemolysis.

During the sampling, blood was transferred from the syringes into the tubes until 4 to 5 ml. of blood had been collected, at which time the tube was transferred to a wire rack in a wide-mouthed vacuum bottle containing crushed ice. After about 3 hours of collecting, tubes of blood were placed in a refrigerator and held there until the serum was separated.

Both syringe and needle were washed after use by drawing distilled water into them and forcibly expelling it. This procedure was repeated until they had been washed three times from each of two distilled water containers. After the syringe was washed, it was separated into its component parts and placed in a draining rack.

For the entire series of 16 determinations, approximately 23 ml. of whole blood were needed. Of this, about 20 ml. of blood were used for serum and 3 ml. for whole-blood determinations. Enough blood could usually be obtained from 30 fish to satisfy these requirements. From 4 to 10 hours of fishing were required to collect enough fish to supply 23 ml. of whole blood.

Throughout the experimental period fish were fin clipped at the time blood was taken. These fish could be recognized if taken in later catches. Of the total of approximately 350 fish represented in these experiments, blood was drawn more than once from only 3 fish. Fish taken by electric shock and held in concrete pools were moved to other pools after blood was drawn from them.

Sampling schedule

Blood samples were taken according to the following schedule:

Sample I	June 24, 1958
Sample II	July 5, 1958
Sample III	July 24, 1958
Sample IV	August 2, 1958
Sample V	August 18, 1958
Sample VI	August 27, 1958
Sample VII	September 29, 1958
Sample VIII	October 7, 1958
Sample IX	October 31, 1958
Sample X	November 18, 1958

A total of 22 fish (sample X) was collected by electric shock on November 11, 1958, and removed to a small concrete pool at the Cornell University fish hatchery. Pieces of tile pipe were added to the pool to provide cover for the fish in their quiescent state. Since the water temperature in the pool was very similar to the temperature of the normal habitat, no significant error in blood chemistry was introduced as a result of moving the fish from creek to pool, and values obtained from samples taken from those fish were reliable estimates of changes normally taking place in the blood of fish in a quiescent state in the stream. A blood sample was taken from these fish on November 18.

Additional information on sampling dates, numbers of fish in the samples, and water temperatures during the sampling periods is included in table 1.

TREATMENT OF BLOOD

Blood collected for serum determinations was stored in heavy-walled, 12-ml. conical centrifuge tubes until clots expressed most of the serum. The blood of the smallmouth bass coagulates very readily but clots are very loose. Centrifugation soon after clotting resulted in very poor yields of serum. Also, if centrifugation was carried out soon after clotting, the serum was very difficult to pick up, even with a micropipette, without stirring up cells and transferring them with the serum. To overcome this problem, coagulation was allowed to continue for 12 to 14 hours after the blood was collected. After this period of time, shrinking of the clots had expressed most of the serum so that centrifugation at approximately 2,500 revolutions per minute produced a maximum yield of serum that could be transferred without disturbing the red cells.

A micropipette with a continuous siphon suction apparatus was used to remove the clear serum into a clean, dry centrifuge tube. The serum was held in a refrigerator until determinations were begun. All serum from a given blood sample period was pooled. Since only a small amount of blood could be taken from individual fish, there was little opportunity to get enough material to make determinations on blood or serum of individual fish. By pooling serum from all fish, rather than having a determination of one component from one or two fish and another component from still another fish, it is possible to get a measurement of each of the 16 components from each fish. This in effect gives us a measurement of a component in approximately 30 fish. There was no evidence of hemolysis in any of the samples. The serum was clear, but with a moderate golden-yellowish cast. Blood collected for whole-blood determinations, treated with an anticoagulant, was held in 12-ml. conical centrifuge tubes under refrigeration until time of analysis.

CHEMICAL DETERMINATIONS

Whole blood from the fish sampled in this experiment was somewhat difficult to handle, even with the use of an anticoagulant. Fibrin clots formed readily unless care was taken in introducing the pipettes or in remixing the plasma and red cells after the blood had been setting in the refrigerator for a few hours. This tendency to form fibrin clots very readily made the use of whole blood or plasma somewhat difficult. For this reason 13 of the 16 determinations were made on serum. Only glucose, iron, and creatinine were determined in whole blood.

The scheme of determinations was designed to measure the more labile components as soon as possible. Protein-free filtrates were prepared when practical and stored in screwcap tubes until analyzed. In general, all 16 components could be determined in a 96-hour period after the blood was collected.

All determinations were made with the use of a Lumetron photo-electric colorimeter, employing methods recommended in the manual of the manufacturer.

BLOOD COMPONENT LEVELS

When this study was undertaken the main objective was to determine normal values for some 16 components in the blood and serum of the smallmouth bass. This information was to be used in a comparative study, or a study comparing the blood composition of several species of fish. It was anticipated that a comparative study of blood composition might provide evidence of the relationship between composition and function in fish, or that habitat requirements (whether salt water, fresh water, and so on), food habits (whether piscivorous, insectivorous, and so on), activity, and other characteristics of a species, might be reflected in blood composition.

Information concerning the blood composition of the normal fish should also be valuable to the fish pathologist. With a series of normal values at hand, it should be possible to describe abnormal, blood-composition patterns characteristic of specific pathological conditions. Characterizations using blood composition should be useful in diagnosing or even predicting disease outbreaks in fish. Information of this type is singularly unavailable to the fishery worker at the present time.

It was anticipated in carrying out determinations periodically that a range of values could be obtained that were normally distributed about a mean or average value; however, it was apparent after the first few series of determinations that the anticipated objective would not be realized. All of the components determined were displaying systematic rather than random variation. Serum total protein and serum globulin levels dropped steadily throughout the first 8 weeks of the experimental period (table 1). During this same interval, serum sodium and potassium increased steadily. All other com-

TABLE 1.—*Concentrations of 16 noncellular components in the blood and serum of the smallmouth bass, measured periodically from June 24 to November 18, 1958*

Item	Samples[1] taken by angling									Sample taken by electroshock
	I	II	III	IV	V	VI	VII	VIII	IX	X[2]
Date blood sample taken	June 24	July 5	July 24	Aug. 2	Aug. 18	Aug. 27	Sept. 29	Oct. 7	Oct. 31	Nov. 18
Number fish in sample	28	26	41	31	30	28	32	31	4	22
Water temperature (F.)	67°	74°	70°	68°	68°	60°	56°	51°	46°	50°
Blood:										
Creatinine (mg%)	0.5	0.8	1.0	0.7	0.5	0.5	0.5	0.6	----------	0.9
Glucose (mg%)	58	58	63	90	68	85	94	100	----------	88
Iron (mg%)	14	17	12	19	18	20	15	17	----------	19
Serum:										
Protein (gm%)	2.6	2.3	2.2	1.9	1.8	2.3	2.4	2.7	3.3	4.5
Albumin (gm%)	1.2	1.2	1.3	1.1	1.1	1.2	0.9	1.4	----------	1.8
Globulin (gm%)	1.4	1.1	0.9	0.8	0.7	1.0	1.5	2.3	----------	2.7
Albumin-globulin ratio	.86	1.09	1.44	1.38	1.57	1.20	.61	.59	----------	.67
Nonprotein nitrogen (mg%)	30.0	36.1	38.4	30.5	28.2	28.6	28.6	28.2	55.9	59.4
Uric acid (mg%)	4.9	3.8	6.0	3.8	6.4	5.4	4.9	3.8	----------	----------
Cholesterol (mg%)	----------	145	145	145	65	176	346	304	----------	300
Calcium (mg%)	----------	12.8	13.8	11.4	12.3	10.2	10.5	12.0	13.2	19.5
Chloride (meq/l)	123	102	111	116	116	128	132	116	127	110
Phosphorus:										
Total (mg%)	89.6	89.3	70.9	61.7	71.4	74.6	76.5	67.5	----------	75.3
Phosphorus, lipid (mg%)	49.3	51.1	45.9	35.2	46.6	46.9	53.0	51.0	----------	61.5
Phosphorus, inorganic (mg%)	16.0	15.0	12.5	12.8	17.3	12.2	9.1	9.4	8.8	9.2
Potassium (mg%)	9.9	6.4	9.0	12.8	17.2	15.0	10.8	8.0	----------	9.0
Sodium (meq/l)	128	120	128	136	148	140	140	140	----------	140

[1] Blood sample removed at time of capture.

[2] Taken on November 11 and held in a concrete pool until the blood sample was taken on November 18.

ponents showed either increasing or decreasing trends, such as these noted.

It became obvious by the end of the experimental period that a normal blood pattern in the smallmouth bass could not be described without including time of sampling in the description. The pattern that was considered normal on July 24 was not the same pattern recorded on August 18 or September 29. It was also obvious that values obtained in these experiments could not be used in comparative studies. The use of a range to describe extreme values of systematic variation is statistically unacceptable, as is calculation of an arithmetical mean from these values. Once this systematic or cyclic variation was noted, the most desirable objective then became an attempt to ascertain the factor or factors responsible for these changes, or an investigation of the metabolism of fish as it is reflected by the blood of the smallmouth bass.

Information on the blood of fish is meager; information on fish metabolism is almost nonexistent. Most of the work published to date consists of measurement data. Data in which concentrations of various components have been measured are readily available, but data in which concentrations of components have been related to metabolism are lacking. Where there is such a lack of data in the literature with which to interpret experimental results, a worker must of necessity weave his findings with the threads of data available on fish, and then attempt to fill in the gaps with information on mammalian metabolism.

Although all components of the blood of the smallmouth bass studied in these experiments are closely related physiologically, a unified presentation of results and discussion would be very difficult. For this reason individual components or groups of closely related components will be discussed first and then in a general discussion the separate parts will be integrated.

SERUM PROTEINS

Information regarding the serum proteins of fish has been slow in accumulating, and that available is limited in its usefulness by the diversity of conditions, species of fish, and analytical methods with which it was obtained. Most of the work on serum proteins has been limited to the comparative approach, i.e., comparing the serum proteins of one species with those of another. Few experiments have been conducted with the aim of relating serum proteins to their function in the life of the fish; however, several papers on the serum and plasma proteins of fish should be mentioned. Lepkovsky (1929) studies the distribution of serum and plasma proteins in several species of teleosts and one elasmobranch. The distribution of blood proteins in the carp and brown trout has been reported by Field et al. (1943). Deutsch and Goodloe, using electrophoresis, in 1945 reported results of studies on the plasma and serum proteins of fish. Deutsch and McShan (1949) conducted electrophoretic studies on the blood of 17 species of fish, and reported differences of taxonomic importance among many of the species. Phillips et al. (1957) reported results of studies on the serum proteins of brook and brown trout. Flemming (1958) has probably made the most exhaustive study of the serum proteins of fish. Using the electrophoretic method, he studied the serum proteins of the carp. He recorded the pattern of the different protein fractions in carp several times during the year, in starved carp, in carp after a winter in ponds, and in carp infected naturally and artificially with carp dropsy or ascites.

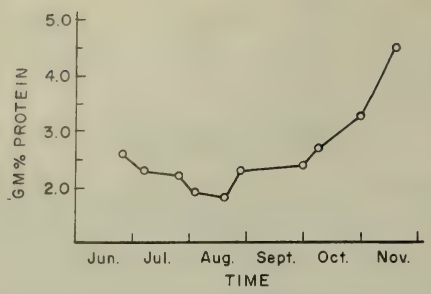

FIGURE 2.—Total protein concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

Serum total protein

Serum protein is a mixture of two proteins, albumin and globulin. These two molecules vary greatly in their physical, chemical, and physiological properties, but together in the blood they are active in regulating the osmotic pressure and, therefore, the water balance of the organism. They transport nutrient materials needed by the anabolic machinery at the cellular level, and mediate the transfer of catabolic products to the sites of excretion. They transport hormones to chemoreceptive centers and perform logistical activities in the defense against bacterial invaders.

In these experiments the concentration of total serum protein has been determined in the serum of the smallmouth bass at intervals from the end of their spawning season in June until the beginning of their quiescent state in November. The results of these determinations are given in table 1, and are shown graphically in figure 2, where concentration in grams of protein per hundred milliliters (gm%) of serum is plotted against time. As is evident from the table and the graph, serum total protein of the smallmouth bass varies considerably with respect to time. From the June 24 level of 2.6 gm% the concentration decreased gradually until it reached a level of 1.8 gm% on August 18. The concentration then increased throughout the remainder of the experimental period, reaching a level of 4.5 gm% on November 18.

Two observations are of interest in these results:

(1) The cyclic nature of the concentration curve is intriguing. If we accept these values as being reliable estimates of changes taking place in the blood of these fish, then we must suppose that the metabolic patterns which maintain serum protein concentrations are cyclic in nature also.

(2) The extent of variation in the serum protein levels is also interesting. In these experiments a decrease of 30 percent in concentration over an 8-week period was followed by an increase of 150 percent in a 12-week period. In higher vertebrates, changes of this magnitude in serum proteins are recorded only after severe trauma, malignancy, or diseases of the kidney. In the smallmouth bass under the conditions of these experiments, such changes apparently are normal.

The possible role of temperature as cause for the cyclic nature of these data should be mentioned at this point. It is possible that the change noted in the concentration curve of serum globulin between June 24 and August 18 was a result of some physiological state induced by rising water temperatures and that the gradually rising concentration curve of the period August 18 to September 29 simply reflected a cooling trend in water temperature. Temperature data collected during each sampling trip (table 1) does not support this "temperature induced" hypothesis. Only in one interval, June 24 to July 5, did a rise in water temperature coincide with a drop in serum globulin levels to support this hypothesis. From June 24 to July 5, the temperature increased from 67° to 74° F. This 7° rise in temperature was accompanied by a decrease in the serum globulin concentration

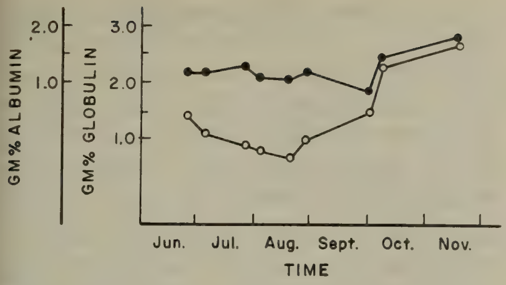

FIGURE 3.—Albumin (solid circles) and globulin (open circles) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958. (Note that scales on the ordinate axes are different.)

from 1.4 gm% to 1.1 gm%; however, the next two decreases (0.9 gm% and 0.8 gm%) are accompanied by two decreases in water temperature (70° and 68° F.). It appears from these data that changes in water temperatures were not responsible for the cyclic changes in the serum globulin concentrations.

Serum globulin

Serum globulin concentrations measured during the 20-week experimental period are recorded in table 1 and are shown graphically in figure 3. This component of the serum decreased from a level of 1.4 gm% on June 24 to 0.7 gm% on August 18. This period of decline was then followed by an increase in concentration to 2.7 gm% on November 18. By comparing these results and the globulin curve with that of total serum protein (fig. 2), it is readily seen that the cyclic nature of the total protein curve is in reality a reflection of changes taking place in the serum globulin fraction. In fact, almost 90 percent of the decrease in total serum protein was due to the loss of serum globulin. From these results then it seems that the cyclic metabolic pattern proposed as being the cause of the changes in total serum protein was in actuality restricted to a cyclic pattern of serum globulin metabolism. That globulin would contribute the major part of the variation in changes of serum total proteins is not surprising. Miller et al. (1949) has shown the half-life of the globulin molecule to be about 3 days, less than half that of albumin. This indicates a very rapid turnover rate or metabolic rate in globulin. This also indicates a more sensitive metabolism, a metabolism that would quickly reflect changes in the catabolic-anabolic relationship. At this rapid rate of turnover, a slight increase in the catabolic rate without an equal increase in the rate of synthesis should result in a decrease in the net amount of globulin. Also, a decrease in energy supply for synthesis and the resulting lowering of the anabolic rate without a decrease in the catabolic rate would have the same effect. An increase in catabolism and a simultaneous decrease in anabolism would rapidly reduce the net amount of globulin. Any one of these three catabolic-anabolic relationships could explain the cyclic nature of the serum globulin concentration observed in these experiments.

Serum albumin

Serum albumin levels of the smallmouth bass changed only slightly during the period June 24 to September 29. Data presented in table 1 and figure 3 show that albumin decreased only 0.1 gm% during the same period that serum globulin levels fell 0.7 gm%. These results are not unexpected. If the evidence concerning the comparative half-lives of the albumin and globulin molecules be applied here, changes in the concentration of serum albumin would be expected only after serum globulin had shown marked changes. However, the explanation does not appear to be quite as simple as has been presented thus far. As seen in figure 3, after September 29 the albumin and globulin curves show identical patterns of

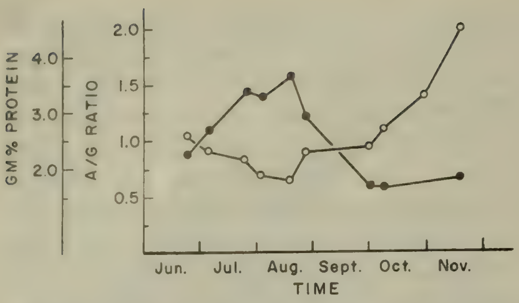

FIGURE 4.—Albumin-globulin ratios (solid circles) and total protein (closed circles) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958. (Note that the scales on the ordinate axes are different.)

increase. Both fractions increase abruptly from September 29 to October 7, then the curves flatten out together until November 18. These curves suggest that some mechanism maintains serum albumin levels when serum globulin levels fall, but that factors favoring a net increase of serum globulin also favor an increase in albumin. Elucidation of this mechanism is not possible with these data.

Albumin-globulin ratio

The albumin-globulin ratios (A/G) during the experimental period showed an inverse relation with serum globulin and serum total protein (fig. 4). This is not unexpected, since serum albumin remained relatively constant throughout most of the experimental period while serum globulin changed markedly. In these experiments the A/G ratio increased from a value of 0.86 on June 24 to 1.57 on August 18. The values then decreased until they attained a more or less stable level after September 29 (table 1 and fig. 4).

The A/G ratio is always greater than 1.0 in mammals. Values approaching 1.0 in man are indicative of impairment of kidney or liver function. Results of determinations of the A/G ratio in fish are confusing. Lepkovsky (1929) reported that the A/G ratio of the menhaden ranged from 1.28 to 2.08, the goosefish from 0.17 to 0.54, the dogfish from 0.09 to 0.35, and the bullhead from 0.20 to 0.36. Field et al. (1943) recorded an A/G ratio of 3.57 for the carp and 2.26 for the brown trout. Phillips et al. (1957) reported an A/G ratio of 2.19 in the brook trout and 0.77 for the brown trout. The significantly different A/G ratios recorded by Phillips et al. (1957) and by Field et al. (1943) for the brown trout (2.26 and 0.77) are tentatively explained by Phillips. He noted the presence of an abundance of diplobacilli which are usually associated with kidney disease in the brown trout. Although typical lesions of the disease were not present, an abundance of the organism could have impaired kidney function enough to bring about excessive loss of albumin in the urine. Dombrowski (1954) reported A/G ratios ranging from 0.53 to 1.23 in carp infected with ascites, a disease characterized by an accumulation of serous fluid in the peritoneal cavity.

On the basis of A/G ratios determined in these experiments, it is possible that differences in A/G ratios reported by other workers are a result of a cyclic relationship. For example, the A/G ratio for the smallmouth bass on June 24 was 0.86, but on August 18 it would have been recorded as 1.57, an increase of about 85 percent. Later in the period an A/G ratio of 0.59 was recorded. These results suggest that the A/G ratio is dependent on the time of year it is determined. This cyclic relationship is probably dependent on several factors characteristic of the yearly life cycle of a particular species of fish. Such things as time of sex-product formation, time of spawning, and response to temperature changes, would affect the timing of the cycle and also the amplitude, but due to the cyclic nature of the causes, the cyclic relationship would prevail throughout the life of the individual.

SERUM NONPROTEIN NITROGEN

The concentration of no other fraction or component of the serum of animals is dependent on as many factors as is nonprotein nitrogen. The concentration of this serum fraction is dependent on the digestion of proteins and the absorption of amino acids, the deamination of amino acids, degradation of the nucleoproteins, the efficiency of the excretory system, and the delicate balance between catabolism and anabolism. Nonprotein nitrogen is truly a fluid record of nitrogen metabolism in the animal body, and yet few components of the serum are more constant. This relative constancy of the whole fraction and the metabolic history which its components record have made nonprotein nitrogen extremely important in clinical diagnosis of pathological conditions. For example, urea levels in man above 10 to 15 milligrams per hundred milliliters of serum (mg%) are indicative of chronic and acute nephritis, metallic poisoning, intestinal obstruction, and some infectious diseases (Hawk et al., 1954). The range of the amino acid nitrogen fraction in the blood of the normal man is 5 to 8 mg%. Values higher than this are indicative of leukemia, acute yellow atrophy of the liver, or severe nephritis (Hawk et al., 1954).

The composition of nonprotein nitrogen in the serum of fish is somewhat different than that in man or other vertebrates. Whereas most of the nitrogen in the nonprotein nitrogen fraction of man is in the form of urea, amino acid nitrogen contributes the bulk of the nonprotein nitrogen in fish. The total concentration of nonprotein nitrogen is remarkably similar in man, the rat, and fish.

The nonprotein nitrogen fraction of fish serum has probably been studied more than any other fraction. Comparative physiologists and taxonomists have found fertile ground for their investigations here. Among the papers available, those by Denis (1913), Wilson and Adolph (1917), Field et al. (1943), and Phillips et al. (1957) are the most rewarding. These authors have partitioned the nonprotein nitrogen of several species of fish into its components; however, as with the serum proteins, little information is available concerning the normal or abnormal metabolism of this fraction and its components.

The experimental scheme for the determination of nonprotein nitrogen was the same as that described for serum protein. In this study, total nonprotein nitrogen has not been completely partitioned. Only total nonprotein nitrogen, creatinine, and uric acid have been determined. The results of these determinations are given in table 1, and are graphically presented in figures 5 and 6, where the concentrations in milligrams per hundred milliliters of serum (mg%) are plotted against time.

Total nonprotein nitrogen

The total nonprotein nitrogen fraction rose sharply from the beginning of the experimental period in June until the latter part of July at which time it declined, returning to its original June level about the middle of August (fig. 5). During this period the concentration rose from 30 mg% and fell to about 28 mg%. From

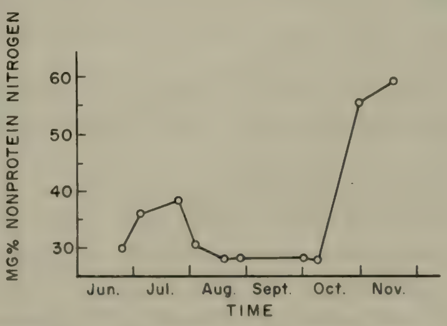

FIGURE 5.—Total nonprotein nitrogen concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

the middle of August until early October the concentration remained constant; however, between early October and the latter part of October it had risen almost 100 percent, to about 56 mg%. The increase continued into November, where at the end of the experimental period the nonprotein nitrogen concentration was about 59 mg%.

According to Field et al. (1943), the nonprotein nitrogen level in the blood of mammals is chiefly determined by the balance among the rates of protein catabolism, amino acid anabolism, and excretion by the kidneys. This same interrelationship probably exists in fish; although the factors contributing to the balance are somewhat different. In fish, the gills are the main nitrogen excretory site and ammonia is the chief nitrogenous excretory product (Smith, 1929); however, as mentioned previously, the largest fraction of nonprotein nitrogen in the blood of fish is contributed by amino acid nitrogen. This suggests a very different pattern of nitrogen metabolism in the fish. For if ammonia is the chief excretory product, yet is not found in large concentrations in the blood, then deamination of the amino acids must take place at the site of excretion—the gills. Also, the presence of large amounts of amino acid nitrogen in the blood suggests some deficiency in the metabolic machinery of the fish, some defect that makes the utilization of amino acids somewhat inefficient.

The machinery of nonprotein nitrogen metabolism appears to be somewhat different in fish than in mammals, but the total effect, that of keeping the nonprotein nitrogen fraction constant, is comparable. This gives us some basis for probing for the explanation of changes in the nonprotein nitrogen in the serum of smallmouth bass noted in these experiments. The increase in serum nonprotein nitrogen from June 24 to July 24 and the gradual return to the June 24 level of about 28 mg% on August 18 probably reflect an increase in the catabolic rate. Of the three basic factors affecting nonprotein nitrogen levels—anabolism, catabolism, and excretion—a change in the catabolic rate of this natural population of fish seems more likely than a change in either anabolism or excretion. According to this assumption, the catabolic rate increased from June 24 to July 24, pouring an added amount of nonprotein nitrogen into the blood stream. Then the catabolic rate returned to its June 24 level on August 18. It will be recalled that this period of increased nonprotein nitrogen levels corresponds to the period of decreasing serum protein levels. It might seem at first glance that the changes in these two components are cause and effect, or that the decrease in serum protein reflects an increased catabolic rate of this fraction. This increased catabolic rate pours an added amount of nitrogen into the blood stream to be excreted, this added amount of nitrogen appearing as nonprotein. Rather than this simple cause and effect relation, evidence will be presented later in support of the postulation that both an increase in nonprotein nitrogen and a decrease in serum protein are effects of the same cause, and that this mechanism brings about a simultaneous increased catabolic rate and reduced anabolic rate.

The period of relative constancy of the nonprotein nitrogen fraction, August 18 to October 7 (see fig. 5), probably reflects stable metabolic relations, relations depicting a positive nitrogen balance. In this metabolic situation, the nitrogen in the nonprotein nitrogen fraction arises primarily from the turnover of endogenous body protein. It is a situation where anabolism is proceeding at a faster pace than is catabolism. That anabolism is increased is evidenced by the increasing serum protein concentration during this period.

Nonprotein nitrogen increased significantly from October 7 to October 31 and remained at that high level until the end of the experimental period on November 18 (table 1 and fig. 5). During this period the concentration increased from approximately 28 mg% to 59 mg%, an increase of more than 100 percent. At first glance this increase appears to be the result of a substantial increase in protein catabolism, and this might be true except that during this period there was a net increase of 1.8 grams of serum protein per 100 milliliters of serum. If the increase in nonprotein nitrogen resulted from an increase in catabolism, apparently it was a selective catabolism, since the serum proteins were not affected. There is another possible explanation for this rapid increase in nonprotein nitrogen. A decrease in the efficiency of the excretory system, such as might have been induced by the quiescent state, could have caused such a rapid rise. Here a more complete partition of the nonprotein nitrogen would have been valuable in explaining this phenomenon. The possibility also exists that both of the aforementioned causes contributed. A combination of increased tissue catabolism brought about by the fasting condition of the quiescent fish together with a decreased efficiency of excretion could account for the increase in nonprotein nitrogen. Morgulis and Edwards (1924) suggested that this combination might be the cause of the increase in nonprotein nitrogen in fasting persons.

Blood creatinine

Blood creatinine, the anhydride of creatine, is the least variable nitrogenous constituent of the blood of mammals (Hawk et al., 1954). Results reported by Wilson and Adolph (1917), Field et al. (1943), and Phillips et al. (1957), indicate that this is also true in the case of fish. Hawk et al. (1954) reports that creatinine in mammals is more readily excreted than is either uric acid or urea, and that even a slight increase in blood creatinine is evidence of marked impairment of kidney function. In contrast with ammonia, which is excreted by the gills, creatinine is excreted by the kidney in fish (Smith, 1929).

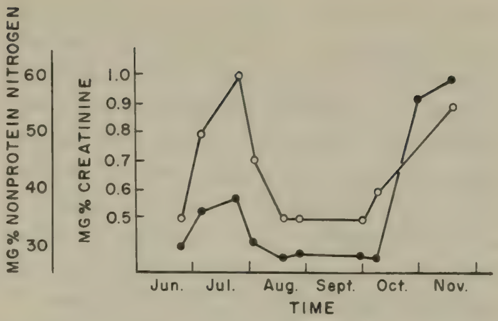

FIGURE 6.—Creatinine (open circles) concentrations in the blood of smallmouth bass, determined periodically from June 24 to November 18, 1958. The serum total nonprotein nitrogen (closed circles) curve from figure 5 is shown for comparative purposes. (Note that the scales on the ordinate axes are different.)

In these experiments, the blood creatinine concentration of smallmouth bass followed closely the pattern previously described for total nonprotein nitrogen. Although the range of concentration was small, the changes in concentration with respect to time are essentially the same as those noted in nonprotein nitrogen. As can be seen from table 1 and figure 6, there was an increase in blood creatinine during the early part of the experimental period, followed by a period of relative constancy, which was followed by a period of increase.

If results obtained in these experiments were examined, using information from mammalian physiology, the two periods of increased creatinine concentration, June 24 to August 18 and October 7 to November 18, would be interpreted as indicating marked impairment of kidney function (Hawk et al., 1954). Apparently, creatinine clearance in the mammalian kidney is so efficient that increased production of creatinine from creatine normally cannot be detected in the blood, so that any increase in creatinine concentration means an impairment of kidney function. Information of this nature is not available for fish; however, it is not likely that kidney dysfunction was the precipitating cause of the increases in creatinine concentration noted in this natural population of smallmouth bass. When interpreted in the light of changes in other blood and serum components, these changes appear to indicate a rather fixed capacity for creatinine clearance, so that increases of creatinine production would be reflected by changes in blood concentration.

According to Fruton and Simmonds (1958), the creatinine excreted in the urine of mammals is correlated with the fate of the muscle creatine, and in conditions characterized by the breakdown of muscle tissues, the excretion of this compound rises markedly. If the assumption is correct that the fish kidney has a rather fixed capacity for creatinine clearance, then an increase in muscle-tissue catabolism would be indicated by an increase in blood creatinine. Interpreted in this light, the two periods of increased creatinine concentration reflect increases in tissue catabolism. This interpretation agrees with the one arrived at previously for total nonprotein nitrogen.

Uric acid

Uric acid has been determined in the blood and serum of fish by several workers, Denis (1913), Vars (1934), Field et al. (1943), Dombrowski (1953), and Phillips et al. (1957). Their results encompass several species of fish, but show very few differences with respect to the species. In fact, different workers report significantly different concentrations from the same species. This is not an unusual situation. Even in homeostatic animals, uric acid determinations are not useful indicators of purine metabolism, either normal or pathological (Hawk et al., 1954).

Uric acid concentrations in the serum of the smallmouth bass as determined in these experiments are recorded in table 1. These results seem to bear no resemblance to patterns observed in total nonprotein nitrogen and creatinine. The tendency for high and low values to alternate during the experimental period is interesting, but with the information available, explanation is difficult.

SERUM PHOSPHORUS

Information concerning phosphorus in the blood and serum of fish is scarce. Only four papers were located in which blood or serum phosphorus or any of its fractions had been determined. McCay (1929) has made the most comprehensive study to date of phosphorus in the blood of fish. He determined partitioned phosphorus concentrations in the whole blood, in the red cells, and in the serum of catfish. In a later paper, McCay (1931) compared the partitioned phosphorus concentrations in the whole blood, in the red cells, and in the plasma of the pike, carp, and bullhead. Field et al. (1943) included serum inorganic phosphorus and serum total phosphorus in the determination of inorganic constituents of carp blood. These same fractions were also determined by Phillips et al. (1956) in the brown trout. From the work of these authors, apparently the serum of fish contains roughly two times as much phosphorus as the serum of man (Hawk et al., 1954). The serum phosphorus fractions, inorganic phosphorus, organic acid-soluble phosphorus, and lipid phosphorus, also appear in fish serum in approximately two times their concentration in man.

In these experiments three phosphorus fractions were determined. Serum total phosphorus, serum lipid phosphorus, and serum inorganic phosphorus have been determined according to the same experimental scheme as the serum proteins, and other components. The results of these determinations are shown in table 1.

Serum total phosphorus

Results of determinations of serum total phosphorus are given in table 1 and are shown graphically by figure 7. From a very high level of almost 90 mg% on June 24 and July 5 the serum total phosphorus level dropped abruptly, reaching a level of about 60 mg% on August 2. From this low point, the levels increased gradually to about 75 mg% on September 29. This was followed by a sharp drop to about 68 mg% on October 7, and then by an increase to about 75 mg% at the end of the experimental period.

One of the most interesting points in these results is the magnitude of the serum total phosphorus levels. Reported levels in other fish have seldom approached even the lowest values noted in these experiments. McCay (1931), discussing differences in the total phosphorus levels of pike, carp, and bullhead plasma, proposed that the differences might be related to the activity of the three species. If high phosphorus levels were associated with activity, then the smallmouth bass used in these experiments would be expected to have extremely high values. However, work by Phillips et al. (1956) on the blood of brown trout, a very active species, tends to discredit the "activity–high phosphorus level" explanation. He reported an average value of 21.1 mg% for the serum total phosphorus concentration in hatchery brown trout, a value about one-third of those recorded in these experiments.

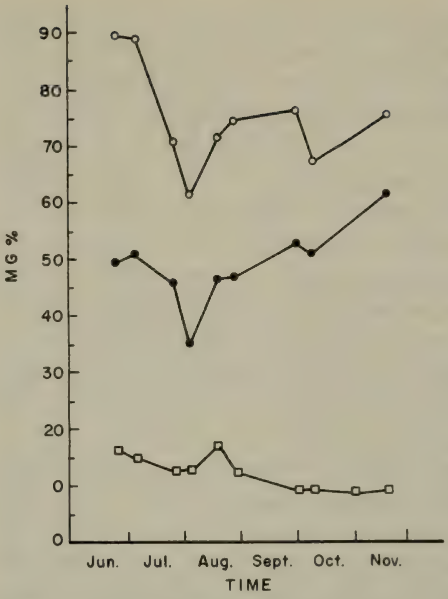

FIGURE 7.—Total phosphorus (open circles), lipid phosphorus (closed circles), and inorganic phosphorus (open squares) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

Serum lipid phosphorus

Experimental results of serum lipid phosphorus determinations are reported in table 1 and are shown graphically in figure 7. Lipid phosphorus values reported here are approximately two times as large as those reported by McCay (1929, 1931) in the carp, pike, and bullhead. The part contributed by serum lipid phosphorus to total phosphorus is demonstrated by a comparison of the two concentration curves (fig. 7). Although the magnitude of the changes are different, the direction of change is always the same.

The most interesting result of these determinations of serum lipid phosphorus lies in the similarity of changes occurring in lipid phosphorus and serum protein (see figs. 2 and 7). Both curves dropped to minimum values in August and then rose more or less steadily to their highest values by the end of the experimental period. Certain differences are important, however. The lipid phosphorus curve reached its lowest point on August 2, while the serum protein reached its lowest value on August 18. Except for the sharp drop in concentration from July 24 to August 2, the relative magnitude of the decrease in lipid phosphorus was not as great as in serum protein. These results suggest that some mechanism producing a net loss of serum protein, and especially of serum globulin, also produced a net loss in serum lipid phosphorus, and that when the effect of this mechanism was reduced, the two components increased in concentration. These results suggest that serum lipid phosphorus was not equally affected, and that only when the causal mechanism was exerting its greatest influence, between August 2 and August 18, was serum lipid phosphorus markedly reduced. This pattern of behavior in the two serum fractions would be expected if energy for synthesis had been systematically reduced during this period.

Both serum protein and serum lipid phosphorus supposedly require energy in the form of high energy phosphate bonds (adenosine triphosphate, ATP) for synthesis. But the synthesis of the serum protein molecule synthesized by specific attachment of ATP-activated amino acids to a nucleic acid template, as suggested by Dounce (1952), Boorsook (1953) and Fruton and Simmonds (1958), would require a great deal more energy (ATP) than would the synthesis of a lipid phosphorus molecule by the "cytidyl–transferase system," in the manner proposed by Smith et al. (1957). If these assumptions are correct, then a reduced energy or ATP supply brought about by a decrease in the rate of energy production would reduce the amount of energy available for all energy-requiring processes including synthesis. Since the synthesis of serum proteins requires a great deal of energy, a shortage in the supply of energy or ATP would be reflected in a lowered rate of production. Synthesis of lipid phosphorus, which represents a relatively small amount of energy, would be much less affected by a decreased supply of energy. Only after the energy supply became seriously reduced would the synthesis of lipid phosphorus be markedly affected.

An energy shortage could explain the changes noted in serum proteins and lipid phosphorus in these experiments. It would suggest that the energy supply available for synthesis in the smallmouth bass decreased during the period from June 24 to sometime between August 2 and August 18. At this point the supply of energy began to increase, bringing about an increased rate of synthesis. This increased synthetic rate is reflected in the net increase of both serum protein and serum lipid phosphorus, which continued to the end of the experimental period.

Serum inorganic phosphorus

Except for the August 18 blood sample, inorganic phosphorus levels showed a general downward trend throughout the experimental period (table 1 and fig. 7). From a level of 16.0 mg% on June 24, the concentration decreased to 12.8 mg% on July 24. This was followed by a sharp rise to 17.3 mg% on August 18. In the blood sample taken on August 27, the inorganic phosphorus level had fallen to 12.2 mg%. By September 29 the concentration had decreased to about 9 mg%, where it remained until November 18.

The downward trend in concentration levels noted in serum inorganic phosphorus was not observed in any of the other 15 components determined. A systematic reduction in concentration was noted in several serum components (globulin, lipid phosphorus, calcium, et cetera), but in all of these cases there was a reversal of trend during the experimental period. For example, serum globulin levels decreased progressively from June 24 to August 18 only to be followed by a progressive increase to the end of the experimental period (fig. 3). These results suggest that the inorganic phosphorus levels in the serum of the smallmouth bass are controlled by a different mechanism than are the levels of other components.

The sharp increase in inorganic phosphorus from August 2 to August 18 corresponds with the period in which globulin levels reached their lowest value, sodium and potassium levels reached their peak, and the energy supply in the smallmouth bass was at its lowest level. When these data are considered along with the discussion in the previous paragraph, it seems possible that serum inorganic phosphorus levels are controlled by a different mechanism than are the other components; that this mechanism is not affected in a similar manner by changes in the metabolism of fish; but that this mechanism may be affected by extreme changes in the anabolic–catabolic balance, such as the condition prevailing between August 2 and August 18 of the experimental period.

SERUM ELECTROLYTES

Electrolytes are distributed in solution throughout all the body fluids, blood lymph, intracellular fluids, digestive juices, and urine. The cations of the electrolytes are the ions of sodium, potassium, calcium, and magnesium, with sodium and potassium predominating. Quantitatively, the chief cation of serum and other extracellular fluids is sodium, while the chief cation of intracellular fluid is potassium. The chief anions of serum and other extracellular fluids are the chloride ion and the bicarbonate ion (West, 1957).

The electrolytes of body fluids have various functions, the most important of which are to contribute a majority of the osmotically active particles, to provide buffer systems and mechanisms for the regulation of pH (acid-base balance), and to give the proper ionic balance for normal neuromuscular irritability and tissue function (West, 1957).

The electrolytes of the animal body are important not only qualitatively but also quantitatively. The distribution of the different electrolytes between intracellular and extracellular fluids is also important. In man the normal serum potassium concentration ranges from 4.0 to 5.4 milliequivalents per liter (meq/l). If this concentration increases to 7 meq/l, characteristic electrocardiographic disturbances are noted. In concentrations above 10 meq/l, the heart may stop in diastole.

The electrolytes of the animal body also perform other important functions. The role of calcium in the clotting of blood and in bone formation is well known. Magnesium is needed for the activation of certain enzymes, such as the phosphatases and transphosphorylases. Sodium and potassium are also necessary in the activation of certain enzymes.

A great deal of information is available regarding the electrolytes of the physiological fluids of fish. Most of this information concerns the role of these ions in osmoregulation. Krogh's work (1939) on the osmotic regulation in aquatic animals is a classical work on this subject. Baldwin (1949) also contributed valuable information on the role of ions in the blood of aquatic organisms. Experiments by Phillips and his group have contributed immensely to information on the role of the calcium ion in osmoregulation (Phillips et al., 1952, 1953, 1954, 1955, 1956, 1957). Compared with this large amount of work on the effect of ions on osmoregulation, little information is available on the role played by the electrolytes in acid-base balance, normal-tissue function, and neuromuscular irritability in the fish.

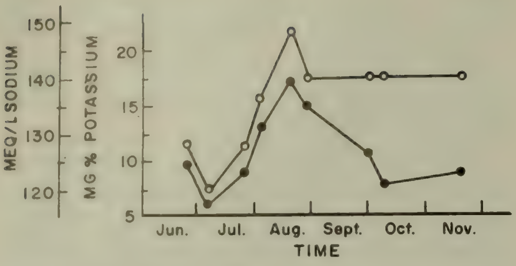

FIGURE 8.—Sodium (open circles) and potassium (closed circles) concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958. (Note that the scales on the ordinate axes are different.)

Serum sodium

Serum sodium concentrations, except for an initial decrease, rose markedly throughout the period June 24 to August 18 (table 1 and fig. 8). From a level of 128 meq/l on June 24, the serum sodium level decreased slightly to 120 meq/l on July 5. Following this decline the concentration rose to a maximum value of 148 meq/l on August 18. On August 24, the serum sodium level decreased slightly to 140 meq/l, where it remained until the end of the experimental period.

Serum sodium levels in the normal man are fairly constant, 130–144 meq/l (Hawk et al., 1954). Work by Field et al. (1943) reported a range of 10 meq/l (127–137 meq/l) in the serum sodium levels of the carp, while Phillips et al. (1956) reported a range of 11 meq/l (151–162 meq/l) in the brown trout. In the smallmouth bass, the range of the seasonal variation in the pooled serum sodium values was 28 meq/l (120–148 meq/l). The values of Field et al. and Phillips et al. are not strictly comparable with the values in these experiments, however, since their work was conducted over a shorter period of time.

The homeostatic mechanism for maintaining serum sodium levels seems to be almost as effective in fish as in higher vertebrates. Results of these present experiments suggest, however, that this mechanism in fish is not capable of maintaining a constant level over a period of several months. The cyclic behavior of the regulatory or homeostatic mechanisms in the smallmouth bass has been suggested by evidence in several previous sections of this paper, and seemingly here is another case in evidence. Apparently the needs of the fish change with respect to the amount of the electrolyte sodium, and the homeostatic mechanism seems to be readjusted to these needs.

An interesting aspect of these data is shown by comparing the curves of serum protein (fig. 2) with the serum sodium curve (fig. 8). On August 18, serum protein reached its lowest recorded level and on this same sampling date serum sodium reached its maximum concentration. These two serum components displayed inverse patterns of behavior throughout the period July 5 to August 27. During this period, a decrease in the one component was accompanied by an increase in the other. This relation was disrupted after August 27 and the sodium concentration remained constant throughout the remainder of the experimental period, even though serum protein increased significantly.

Serum potassium

As shown in figure 8, serum potassium concentrations behave in a manner similar to serum sodium. Although the magnitude of changes were different in the two components, the trends in variation were identical from the beginning of the experimental period until August 27. The serum potassium at the beginning of the experimental period was 9.9 mg% (table 1). After a decrease to 6.4 mg%, the concentration increased steadily through four sampling dates until it reached a level of 17.2 mg% on August 18. This rapid increase was followed by a decrease in concentration until October 7. At the end of the experiment, the concentration was only slightly lower than the concentration at the beginning of the experiment.

The relation between serum sodium and serum potassium concentrations in these experiments should be mentioned. Changes in the serum sodium and the serum potassium concentrations between consecutive sampling dates are shown in table 2. Also recorded are the ratios of those changes. For example, between June 24 and July 5 the sodium concentration decreased 18 mg%. During the same period, serum potassium decreased 3.5 mg%. Here the decrease in sodium was 18/3.5, or 5.1 times as great as the potassium decrease. Between the sampling dates, July 5 to July 24, sodium increased 18 mg% while serum potassium increased 2.6 mg%. The increase in sodium was 18/2.6, or 6.9 times as great as the increase in potassium. In the next interval, July 24 to August 2, the sodium increase was 19/3.8, or 5 times as great as the potassium increase. As shown in table 2 and suggested by figure 8, any change in sodium concentration, increase or decrease, is accompanied by a similar change in serum potassium, but with approximately one-sixth of the magnitude.

The way in which the information on the relationship of serum sodium and serum potassium has been presented is not meant to imply that a change in the concentration of potassium resulted from a change in the sodium concentration, or that the reverse relationship was true. Although either of these relationships might occur, the ratio employed was simply the easier method, in that ratios calculated in this manner are whole numbers rather than small decimal fractions.

The relation between the levels of serum sodium and serum potassium as presented in table 2 does not explain why these two electrolytes increased in concentration during the period that serum protein decreased. Nor does it explain why the electrolytes reached their maximum concentration on August 18, the sampling date that the serum protein concentration was at its lowest point. However, this relationship does suggest that despite the magnitude of the changes demonstrated, some homeostatic device has been active in maintaining the correct balance between these two important electrolytes, in maintaining a correct sodium–potassium ratio.

TABLE 2.—*Change and ratio of change of serum sodium and potassium concentrations between sampling dates*

Blood sample number	Date of sample	Change in serum concentration (in mg%) of—		Ratio of change
		Sodium	Potassium	
I	June 24, 1958			
		[1] 18	[1] 3.5	5.1:1
II	July 5, 1958			
		18	2.6	6.9:1
III	July 24, 1958			
		19	3.8	5.0:1
IV	August 2, 1958			
		27	4.4	6.1:1
V	August 18, 1958			
		[1] 18	[1] 2.2	8.2:1
VI	August 27, 1958			

[1] Concentration decreased between these 2 sampling dates.

There are two possible explanations for the abrupt rise in the serum sodium and potassium curves (fig. 8) during the period when the serum protein and serum lipid phosphorus concentrations were decreasing. First, in the discussion of the nonprotein nitrogen fraction, an increased catabolic rate was suggested as the reason for the increased nonprotein nitrogen levels from June 24 to August 18. It was also suggested as a partial cause for the decrease in serum protein. If this assumption is correct and the increase in the catabolic rate were great enough to initiate tissue catabolism, potassium would be released into the plasma as a result of cellular disruption. A negative potassium balance associated with abnormal destruction of body tissue in mammals has been discussed by West (1957) and Hawk et al.

(1954). According to this proposition, the increase in serum potassium caused by catabolism of tissue would be accompanied by an increase in serum sodium as a result of activity on the part of the homeostatic device responsible for maintaining the correct sodium-potassium ratio. Once the tissue catabolism ceases, as evidenced by the return to normal of the nonprotein nitrogen curve on August 18 (fig. 5), the potassium levels fall.

The second possible explanation of the rapid increase in serum sodium and potassium concentrations concerns the adjusting of osmotic pressure of the serum or plasma. West (1957) discusses the increased rate of reabsorption of sodium salts in the distal tubules of the kidney as a method whereby the mammalian body can correct conditions in which electrolyte concentration and osmotic pressure of plasma fall below normal. In addition, he notes that this condition is also alleviated by excretion of a more dilute urine, thereby concentrating the plasma somewhat and increasing the osmotic pressure. The situation in the blood of the bass is not exactly comparable with the one that West describes, in that the fish apparently has no electrolyte deficit. It is assumed, however, that as a result of the decrease in serum protein there must be a decrease in osmotic pressure in the bass. It is possible, then, that the increase in serum electrolytes is a compensatory activity on the part of the homeostatic mechanism of the fish to maintain the total number of osmotically active particles in the plasma despite a net loss of serum protein molecules. Increase in the total number of osmotically active particles is possibly brought about by increased active transport of the sodium and potassium ions across the gill membranes into the body (Krogh, 1939). An increase of electrolytes in the plasma could be even more deadly than the loss in osmotic pressure if the different electrolytes were not present in the proper amounts. In this case, even though the concentration of both sodium and potassium have increased, these increases have been maintained in physiological balance (table 2). This was pointed out in the discussion on the sodium–potassium relation.

Serum calcium

Calcium is an essential constituent of all living cells. Its mode of action is not clear, but it appears to play a part in decreasing the permeability of cell membranes. Its effect on neuromuscular mechanisms is shown in higher animals by the development of hyperirritability and tetany as a result of a decline in calcium content of the blood (Hawk et al., 1954). Calcium is apparently necessary in activation of the adenosine triphosphatase activity of myosin, but may uncouple oxidative phosphorylation when present in excess (Fruton and Simmonds, 1958).

In fish, the calcium ion plays an important role in osmoregulation. Phillips et al. (1954) have shown that calcium is absorbed from the water by small, nonfeeding brook trout, and that the absorbed calcium is deposited in body tissues. His group has also shown highly significant differences in the amounts distributed between body parts. Other experiments by this group show that the rate of distribution of calcium to the body parts is a function of the metabolic activity of the trout. They have also shown that the metabolic activity is dependent to some extent on the physical composition of the water. In a later work, Phillips et al. (1957) have shown that the concentration of calcium in the water significantly affects the utilization of calcium from food. More calcium was absorbed by the brook trout from its food in water containing 5.0 parts per million (p.p.m.) calcium than from waters containing 50.0 p.p.m.

Discussing the management implications of calcium in osmoregulation, Phillips et al. (1956) suggested that since energy must be expended by the fish to maintain osmotic equilibrium, the increased metabolic activity on the part of the fish in low-calcium waters could possibly influence the growth of fish. The increased utilization of energy to maintain osmotic equilibrium would leave less energy available for anabolism or growth. In support of this he cites work by Reimers et al. (1955) that showed an apparent positive correlation between calcium content of alpine lake waters and the growth of brook trout.

As is evident from the previous section, there has been a great deal of critical work on the osmoregulatory effect of calcium as well as the effect of changes in calcium concentration on the metabolic rate, but there is little information available on what part this ion plays in metabolism or what changing blood levels mean in terms of changing metabolic patterns. In these experiments, changes in the serum levels of calcium were pronounced (table 1 and fig. 9). Although variation in serum calcium was more erratic, at least during part of the experimental period, it nevertheless demonstrated cyclic tendencies. The calcium concentration during the period July 5 to August 27 was characterized by alternate increases and decreases, but it demonstrated an overall decrease. Following a period of about 4 weeks (August 27 to September 29) in which the concentration remained very nearly the same, the levels began to increase, finally reaching a level of almost 20 mg% at the end of the experimental period.

The behavior of the calcium concentration is very difficult to correlate with behavior of any of the other components. Although it tended to behave generally like serum protein, the relation is not so clear as the relation between protein and sodium or protein and potassium. According to Hawk et al. (1954), there is a reasonably close relation between serum calcium, inorganic phosphate, and protein in man. The relation can be expressed by a multiple-regression equation. This relation does not seem to exist in the smallmouth bass. Although the correlation of serum calcium levels with serum protein levels would probably be significant, the serum inorganic phosphorus levels seem to be totally unrelated to either. Serum calcium levels in these experiments do seem to be cyclic in nature, but with the present state of information on factors affecting the regulation of this electrolyte in the blood, no explanation can be offered for its behavior.

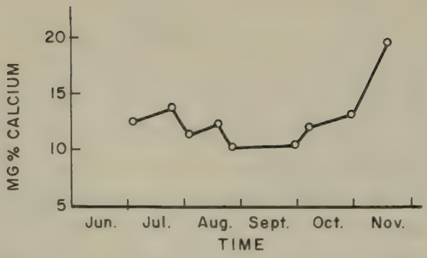

FIGURE 9.—Calcium concentrations in the serum of smallmouth bass, determined periodically from July 5 to November 18, 1958.

Serum chloride

The chloride ion is intimately involved in several physiological processes of the fish. In addition to being the chief anion of both gastric juice and serum, the chloride ion maintains acid-base balance by its participation in the "chloride shift," and it also contributes a large number of osmotically active particles to the process of osmoregulation (Davson, 1951). Krogh (1939) has shown that an active chloride-absorbing mechanism in the gill compensates for the continual loss of the ion in the copious, dilute urine of fish. He also found that the ability to absorb chloride and the threshold of chloride concentra-

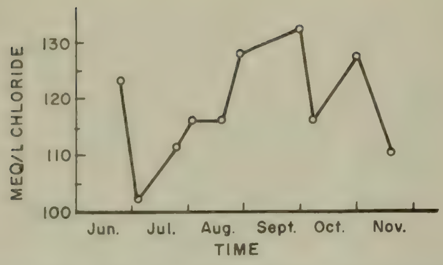

FIGURE 10.—Chloride concentrations in the serum of smallmouth bass, determined periodically from June 24 to November 18, 1958.

tion of the water from which the ion could be absorbed varied with the species.

In these experiments considerable changes were noted in the chloride concentration of the serum of the smallmouth bass. The results of the determinations on serum chloride are given in table 1 and are shown in fig. 10. After a sharp drop from 123 meq/l to 102 meq/l, serum chloride increased throughout the period July 5 to September 29, finally reaching a high of 132 meq/l on September 29. This increase was followed by a series of decreases and increases with the level at the end of the experiment being 110 meq/l. Here, as in the calcium concentration curve, there seems to be little similarity between the pattern of changes of serum chloride concentration and other constituents of the serum. Although there seems to be a cyclic nature in the behavior of this component, the timing of the cycle seems to be completely unrelated to the timing of other apparently cyclic constituents. Unfortunately, even with the amount of information available concerning the absorption and excretion of chloride, little is known about the mechanism by which these processes are controlled. The changes observed in the serum chloride concentration undoubtedly are related to the changes in the anabolic-catabolic relationship, which has been suggested as being the cause for the behavior of other components of the serum.

SERUM CHOLESTEROL

Cholesterol is an important component of living systems. It belongs to a group of crystalline alcohols known as the sterols. It apparently holds a key and central position in the metabolism of many closely related and biologically important compounds. In recent years cholesterol has come under increased scrutiny due to its possible role in cardiopathy. End products of the metabolism of cholesterol are many and include the bile acids and the varied group of steroid hormones. Almost all of the cholesterol in human serum (free and esterified) is associated with the lipoproteins (Fruton and Simmonds, 1958).

Very little information is available on cholesterol metabolism in fish. Field et al. (1943) have noted that cholesterol accounted for more than half of the total lipids found in carp blood. He also found almost one-half the cholesterol in the free state. Dombrowski (1954) reported that the cholesterol level in the blood of carp infected with carp dropsy or ascites was significantly lower than the level in healthy carp. Phillips et al. (1957) reported slightly lower values for brook trout than for brown trout.

Cholesterol levels in the serum of the smallmouth bass were subject to a great deal of variation. Figure 11 demonstrates the extent of variation encountered.

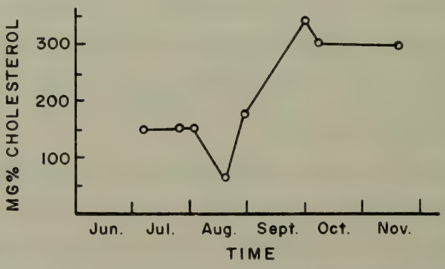

FIGURE 11.—Cholesterol concentrations in the serum of smallmouth bass, determined periodically from July 5 to November 18, 1958.

After recording three successive readings of 145 mg% from July 5 to August 2, the cholesterol level dropped more than 100 percent to a level of 65 mg% on August 18 (table 1). This drop was followed by a dramatic rise to 346 mg% on September 29. Levels greater than 300 mg% were maintained throughout the remainder of the experimental period.

Here again the metabolic patterns producing changes in serum levels of other components seemingly are at work, but unlike serum calcium and chloride, changes in serum cholesterol seem to be more directly related to variation noted in serum globulin, serum potassium, et cetera. Serum cholesterol remained low throughout the period that serum protein levels were decreasing, and reached its lowest level on August 18, which will be remembered as the date corresponding with a minimum value in serum protein, the return to apparently normal levels of nonprotein nitrogen, and maximum values in sodium, potassium, and inorganic phosphorus. The levels of serum cholesterol from the beginning of the experimental period to August 18 were extremely low compared with values obtained in other fish. Field et al. (1943) reported a range of 244 mg% to 364 mg% free cholesterol in the whole blood of carp. Phillips et al. (1957) found an average of 316 mg% cholesterol in the serum of the brook trout and 402 mg% cholesterol in the serum of the brown trout. Apparently, then, the smallmouth bass were actually in a state of hypocholesterolemia from July 5 to August 18.

In man, decreased cholesterol or hypocholesterolemia is found in conditions of hyperthyroidism, pernicious anemia, and certain types of liver disease (Hawk et al., 1954). Also, almost all of the cholesterol of human plasma is associated with lipoproteins, and these lipoproteins are identified electrophoretically with certain globulin fractions (Oncley, 1956). Here, then, are two possible explanations for the low cholesterol values:

(1) A condition of hyperthyroidism during the period July 5 to August 18 could account for the low values; or (2) it is conceivable that since cholesterol is so intimately associated with globulin that an interference in globulin metabolism might have affected serum cholesterol levels. It is apparent from figure 3 and previous discussions that globulin metabolism was interfered with during this period.

BLOOD GLUCOSE

The following discussion provides little background for the interpretation of blood glucose data in these experiments and may seem to be more detailed than is justified by available information; however, such a discussion is necessary to formulate an explanation of the possible role of energy metabolism in producing the blood composition patterns observed in the smallmouth bass. Since glucose and other carbohydrates are intimately related to energy metabolism, the discussion of carbohydrates is included in this section.

The role of blood glucose in mammalian metabolism is well known. It plays the same type role in the metabolism of fish, but certain experimental evidence suggests that its role in normal fish metabolism is more restricted than in mammals. Phillips has contributed a significant amount of work on this subject by publication of a paper on carbohydrate utilization in trout (Phillips et al., 1948). He noted that blood glucose levels of trout increased after ingestion of glucose, just as in mammals. However, the curve of the rise and fall of the blood sugar following the feeding of glucose closely resembled the curves found in studies of the diabetic man. He further noted that insulin injections quickly lowered the blood sugar after it had been raised by glucose feeding. Phillips concluded that trout are unable to use urine clearance as a means of eliminating overdoses of ingested glucose when he could find no evidence of carbohydrate excretion. Feeding carbohydrates over long periods was shown to cause pathological storage of glycogen and was strongly correlated with increased mortality. On the basis of this work, Phillips suggested that the diet of trout should consist of no more than 9 percent digestible carbohydrate or that the fish should not be fed more than 4.5 grams of carbohydrate per kilogram of body weight per day.

From these experimental results it seems that fish lack the metabolic machinery to handle high levels of carbohydrate, and that this deficiency is probably due to reduced insulin supply resulting from a lack of "Islets of Langerhans" tissues; however, Strand (1958) suggested that pathological storage of glycogen could be prevented in salmon and brook trout by adding epinephrine to the diet. Epinephrine, or adrenalin, accelerates the conversion of glycogen to hexose phosphates by promoting the conversion of inactive phosphorylase to the active enzyme (Fruton and Simmonds, 1958). Apparently the inability of fish to utilize carbohydrates arises not only from a shortage of insulin, but also from the lack of a mechanism to mobilize carbohydrate reserves.

In mammals, most of the energy for metabolism is derived from the oxidation of glucose to carbon dioxide and water; but in fish this energy-producing mechanism is seriously impeded by an inability to metabolize glucose. Thus, the fish must depend on either fat or protein as a source of energy; however, oxidation of fats requires the proper functioning of the citric acid cycle. From the discussion by Haurowitz (1955) it is evident that maintenance of the citric acid cycle is dependent on carbohydrate metabolism, since efficient regeneration of oxalacetic acid in the cycle is dependent on the degradation of glucose. Further evidence of the dependency of proper fat metabolism on normal carbohydrate metabolism is shown by the appearance of symptoms of incomplete fat oxidation (ketosis) in diabetes (White et al., 1954).

From the foregoing discussion it seems likely that metabolism of fats is dependent on the metabolism of carbohydrates, and since carbohydrate metabolism in fish is inefficient, normal utilization of fats or the ability to handle high levels of dietary fat is also reduced. Evidence in support of this premise has been reported by Phillips and Podoliak (1957). Their experiments show that high dietary levels of soft fat produce edema in trout, and that high-fat diets may also cause fatty infiltration of the liver. Excessive mortalities occurred among trout with extreme fatty infiltration of the liver.

Since carbohydrate and fat metabolism are seemingly inefficient in the fish, the only other source of large amounts of energy is protein. This need for protein as a source of energy is reflected in the high levels of protein in the diet of hatchery trout. The generally accepted level of protein in trout diets is 28 percent. DeLong et al. (1958) have reported that protein levels in the diet of salmonids should be in the neighborhood of 47 percent. These values are extremely high when compared with levels in the diets of other animals. Recent work by Phillips et al. (1957) suggests that this high level of protein can be reduced by proper attention to calorie balance in the diet.

From available information, it appears that fish must utilize a higher proportion of protein for energy than must other animals. This is probably of little consequence in the normal metabolism of the fish or during a period when there is energy expenditure only for maintenance, some growth, osmoregulation, et cetera. However, this need could have a profound effect on the metabolism of the fish during periods when a great deal of energy is required for synthesis or by an increased metabolic rate. Such needs would occur during formation of sex products, during a period of abnormally high water temperatures, or as a result of an elevated metabolic rate such as would be brought about by increased thyroid activity. In periods of abnormally high energy requirements, it is even conceivable that energy intake would not be high enough to meet the demands and actual tissue catabolism would take place.

Results of blood glucose determination are given in table 1 and are shown in figure 12. Except for one erratic value the blood glucose level increased throughout the experimental period until October 7 and decreased slightly between October 7 and November 18. Beginning at 58 mg% on June 24, the blood glucose concentration rose to 63 mg% on July 24. A rather erratic increase to 90 mg% from June 24 and August 2 was followed by a similar decrease to 68 mg% on August 18. From this point, blood glucose levels increased to 100 mg% on October 7. The level decreased slightly to 88 mg% at the end of the experimental period.

The increase in levels of blood glucose during the experimental period can possibly be explained by a decrease in rate of insulin production. This would mean that from June 24 to July 24 an abnormally high level of insulin was promoting a very rapid utilization of blood glucose, thereby keeping the glucose levels low. Fruton and Simmonds (1958) have suggested that thyroxine may stimulate insulin production. High levels of thyroxine as a result of hyperthyroid activity have already been proposed as a possible cause for the low levels of serum cholesterol noted during this period. Assuming this general hypothesis to be correct, a gradual decline in thyroid activity after July 24 with its attendant decline in the stimulation of insulin production, would result in a rise in blood sugar; however, this does not explain the rapid increase from 63 mg% to 90 mg% and the decrease to 68 mg% between July 24 and August 18.

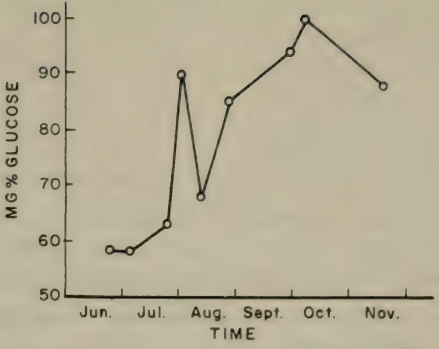

FIGURE 12.—Glucose concentrations in the blood of smallmouth bass, determined periodically from June 24 to November 18, 1958.

Another possible explanation for the changes in blood glucose levels in the bass could have been a decrease in the effectiveness of insulin during this period. In mammals, the adrenal corticoids (the interrenal corticoids in fish) have been shown to be antagonistic to insulin (Long et al., 1940; Fruton and Simmonds, 1958). This effect is apparently accomplished by producing a hyperglycemia by gluconeogenesis without affecting the insulin supply directly. This increase in glucose is accompanied by an increase in nitrogen excreted, suggesting that the glucose increase is at the expense of protein. This proposal probably explains less of the observed facts in the blood glucose pattern than the hyperthyroid hypothesis, but it does possibly explain one perplexing bit of information—the erratic behavior of the glucose curve between July 24 and August 18 (fig. 12). The studies of Brodish and Long (1956) support the hypothesis that adrenal cortical trophic hormone (ACTH) is released by a biphasic mechanism—a rapid release of the hormone under neural control followed by a prolonged release phase indicative of humoral regulation. If this study could be applied to fish, a biphasic release of the trophic hormone by the pituitary, bringing about a biphasic production of the interrenal corticoids, would possibly explain the biphasic increase in blood glucose. According to this proposal, the sharp rise and fall of the glucose curve from July 24 to August 18 was a result of gluconeogenesis caused by the first phase of the biphasic release of the interrenal corticoids. The more gradual rise of the glucose curve from August 18 to October 7 would correspond with the second phase of interrenal corticoid release.

BLOOD IRON

Iron exists in the body chiefly in the heme portion of the hemoglobin of the red blood cells. It is also found in plasma in combination with serum globulin and is an indispensable constituent of various oxidation-reduction enzymes.

Results of iron determinations on the blood of the smallmouth bass are given in table 1. These values demonstrate a considerable amount of variation in blood iron during the experimental period, and since most of the iron in blood is present in hemoglobin, it is apparent that hemoglobin concentrations varied considerably. Comparatively speaking, the blood of the smallmouth bass has a rather low iron content. Hall et al. (1926) reported an average value of 32.9 mg% blood iron in the menhaden. Field et al. (1943) reported an average value of 31.5 mg% blood iron (recalculated from percent hemoglobin) in the carp and an average of 25.5 mg% in the brown trout. Phillips et al. (1956) reported an average of 29.4 mg% blood iron in the brown trout. In the small mouth bass, blood iron values ranged from 12 mg% to 20 mg%, with little evidence of any systematic variation.

There is little similarity between the changes noted in blood iron and in any of the other components of the blood or serum of the smallmouth bass. Apparently, the metabolism of hemoglobin and iron is not subject to the same cyclic pattern of variation noted in the other components.

METABOLISM IN SMALLMOUTH BASS

Throughout the separate discussions in previous sections, possible explanations for experimental results obtained in this study have been proposed, but no attempt has been made to integrate these proposals. In this discussion, an attempt will be made to collect the proposed explanations into a metabolic picture.

In attempting to integrate the several discussions, the experimental results will be considered by periods.

PERIOD 1—JUNE 24 TO AUGUST 18

In the separate discussions, the following proposals have been made to explain the observed changes between June 24 and August 18:

1. Gradual decrease in serum total protein and serum globulin—caused by an increase in the catabolic rate of metabolism coupled with a decrease in the anabolic rate.

2. Increased levels of nonprotein nitrogen—resulted from an increase in protein catabolism.

3. Blood creatinine increase—muscle-tissue catabolism.

4. Lower levels of lipid phosphorus, especially between July 24 and August 2—decrease in anabolic rate.

5. Increase in serum sodium and potassium levels—increase in tissue catabolism or osmotic adjustment to compensate for loss of serum proteins.

6. Low serum cholesterol levels—hyperthyroidism or interference with globulin metabolism.

7. Relatively low glucose levels—high insulin level caused by hyperthyroidism.

It has been suggested in five of these seven components that a decrease in synthesis or anabolism coupled with an increase in destruction or catabolism would explain the data obtained in these experiments. Hyperthyroidism has been suggested as the cause of the observed results in the two other cases. The suggestion that synthesis would be reduced during this period and degradation increased seems unrealistic. During this period, water temperatures are usually nearing their summer peak and food should be readily available. It is difficult to understand how there could be a shortage of anything needed by the metabolic machinery of the smallmouth bass, at least not enough of a shortage to promote tissue catabolism.

A reduced energy supply as a result of hyperthyroid function could explain all of the observed changes described. Thyroxine, the hormone produced by the thyroid gland, uncouples oxidative phosphorylation from cellular respiration or electron transport thereby speeding up the rate at which oxidation takes place, but producing less energy in the form of ATP in the process (Maley and Lardy, 1955). Several studies on the thyroid of fish have demonstrated cyclic changes in the functioning of that gland, and several investigators have presented histological evidence of an increase in the spring of the year (Brown, 1957). Barrington and Matty (1954) found maximum thyroid activity in the minnow, *Phoxinus*, between February and May. Swift (1955) demonstrated that thyroid activity in the brown trout reached a peak in early July, and he also noted a decreased rate of growth during this period.

Peters and Van Slyke (1946) have further suggested that thyroxine causes no actual destruction of protein, but merely increases demands of the body for fuel. That fish apparently must normally utilize some protein for energy has previously been discussed, so, in hyperthyroidism, the fish would be faced with an increased demand for fuel and little other than protein to satisfy this demand. It is conceivable that the demand for protein to be used for energy could become great enough to bring about some tissue catabolism. An increase in tissue catabolism and an attendant increase in blood nonprotein nitrogen as a result of high thyroxine levels have been discussed by Peters and Van Slyke (1946).

In summary, a proposed integrated picture of metabolism for period 1 (from June 24 to August 18) would be as follows:

Increasing thyroxine levels caused by hyperthyroid function in the smallmouth bass increased the metabolic rate and at the same time reduced the amount of energy produced. With less energy available, the synthesis of serum proteins was reduced, causing a decrease in their net concentration. As a result of the increase in metabolic rate, more energy was also required. The fish could not efficiently utilize carbohydrate or fat to provide this energy, so the tissue proteins were called on to provide amino acids for energy production. As a result of this increase in tissue catabolism, nonprotein nitrogen and creatinine levels in the blood increased. Lipid phosphorus, requiring less energy for synthesis, was not greatly affected during the early part of this period. With the loss of the osmotic effect of serum protein, some homeostatic device in the fish initiated an increase in serum sodium and potassium levels to maintain the number of osmotically active particles available in the plasma. Hypocholesterolemia, caused by hyperthyroidism was evident throughout this period, and an increase in insulin production brought about by high levels of blood thyroxine kept blood glucose levels low.

As the levels of circulating thyroxine increased toward the latter part of period 1, reducing even more the energy supply available for metabolism, the trends just noted would continue, but lipid phosphorus levels would also begin to show the effect of increased hyperthyroidism, finally falling rapidly between August 2 and August 18. Cholesterol levels, which have been low throughout the period, would go even lower as hyperthyroidism increased. The blood glucose concentration increased sharply from July 24 to August 2 only to return to about the July 24 level on August 18, probably as a result of the first stage in the biphasic release of corticoid hormones, proposed in the discussion of blood glucose. Sometime between July 24 and August 2, the increase in thyroid function began to abate. The levels of thyroxine were still high, but the rate of increase was not so great. By August 18, thyroxine levels had reached their highest point as was evidenced by the increase in serum protein after this time.

PERIOD 2—AUGUST 18 TO OCTOBER 7

Concentrations of serum components between August 18 and October 7 simply reflected a return to a pattern of metabolism characterized by a lower metabolic rate. Thyroxine levels were decreasing during this period and energy for synthesis was becoming readily available. Tissue catabolism was no longer needed to supply amino acids for energy production. As the protein in the plasma increased, the number of other active osmotic particles needed was reduced, so that sodium and potassium levels were lowered. Cholesterol levels freed from the influence of hyperthyroidism were beginning to rise, and glucose levels increased rapidly, probably because of the second stage in the biphasic release of gluconeogenic corticoid hormones.

PERIOD 3—OCTOBER 7 TO NOVEMBER 18

The period was characterized by a rapid increase in nonprotein nitrogen and creatinine and a moderate increase in serum lipid phosphorus and serum protein. These results would seem to be in complete disagreement with the explanation of the increase in nonprotein nitrogen, which accompanied the decrease in serum total protein during period 1.

To explain the contrasting experimental results shown in the two periods, it is necessary to compare the conditions prevailing in both periods 1 and 3. In period 1 (June 24 to August 18), food was readily available from which metabolism should have been able to produce a favorable energy balance, but due to the hyperthyroid condition, available food was utilized inefficiently, resulting in a negative energy balance and causing an increase in protein catabolism to meet energy needs. This increase in catabolism brought about an increase in serum nonprotein nitrogen and blood creatinine levels.

During period 3 (October 7 to November 18), conditions were somewhat different. By October 7, most of the fish were in the quiescent state, a physiological state characterized by lack of activity and a state in which the fish feed very little, if at all. Also there is evidence that the thyroid of fish is less responsive to the pituitary hormone, thyrotropin, at low temperatures (Pickford and Atz, 1957). This would suggest that the metabolic rate would also be reduced somewhat. Peters and Van Slyke (1946) described elevated blood nonprotein nitrogen levels accompanied by gradually falling urinary nitrogen levels during fasting, which they suggested may be a result of accelerated nitrogen catabolism, decreased excretion, or both. Fasting accompanied by rising nonprotein nitrogen levels and low nitrogen excretion could explain the observed data here, but it is difficult to explain how serum protein synthesis could be progressing during this period of starvation.

The picture presented by nonprotein nitrogen and serum protein increase in the blood of bass in period 3 leads to interesting speculation. This rapid rate of serum protein synthesis during a period when there was no food intake suggests that the amino acids being used in that synthesis were being transported from some other tissue to the liver where the serum proteins were produced. This, then, would be selective catabolism of body protein accompanying selective anabolism, and the high levels of nonprotein nitrogen noted simply reflected a higher rate of amino acid transport for the synthesis of serum proteins.

ROLE OF CYCLIC HORMONAL RELATIONSHIPS IN BLOOD COMPOSITION CHANGES

In the reconstruction of the metabolism proposed in the previous discussions to account for changes in the blood composition of the smallmouth bass from June 24 to November 18, 1958, three general periods of different metabolic activity have been proposed as causing those changes:

(1) Period 1 (June 24 to August 18) was characterized by a reduced anabolic rate, an increased catabolic rate, and an increase in the overall metabolic rate. It was suggested that a hyperthyroid state was responsible for these conditions.

(2) A metabolic pattern in period 2 (August 18 to October 7) suggestive of a relatively lower metabolic rate was evident. During this time catabolism decreased and anabolism increased.

(3) The proposed characterization of metabolism in period 3 (October 7 to November 18) was one of selective catabolism and anabolism, a general lowering of the metabolic rate, and decreased excretion—induced by the quiescent state and the fasting condition associated with it.

In the following discussion an attempt will be made to relate the proposed changes in catabolic-anabolic balance and thyroid function to possible cyclic hormonal relationships.

Several types of cyclic physiological behavior have been noted in fish (Brown, 1957: chapters VI, VII, and IX). Among these types are sex cycles, cyclic growth-rate changes, periods of relative inactivity followed by periods of great activity, and cycles in feeding.

Environmental change has been the cause commonly suggested to explain metabolic cycles in fish, such as growth, activity, and feeding. In general, the metabolism of the fish is thought to be controlled by the environment. Thus, in cold water fish eat little and grow little. In extremely warm water they become listless and inactive. Increases in water temperature, especially from an extremely low to a more moderate level, caused increased feeding activity. Certainly, changes in environment do affect the fish in a very real manner, but apparently environment is not solely concerned. Brown

(1946) has shown that even under constant environmental conditions cyclic metabolic changes take place. In her study, 2-year-old brown trout were kept at 11.5° C. under 12 hours of artificial lighting a day, provided with a constant rate of water flow, and were allowed to eat all they would consume two times a day. She noted significant cyclic changes in growth rate of the trout several times during the experimental period of more than a year, and that they became sexually mature under these conditions. This information suggests metabolic cycles in fish that are active, even under constant environmental conditions, or that environment is not the sole cause of changes in growth rate, feeding response, and activity.

Experiments carried out by Ekberg (1957) also suggest physiological cycles in fish that act independently of environment. He studied respiration in the tissues of goldfish that had been adapted to warm and cold temperatures. In these studies, Ekberg noted that oxygen consumption by gill tissues was greater during the summer months than during the winter months, and that this relation was not affected by adaptation to warm or cold temperatures. Apparently, tissue respiration was affected more by the time of year than by changes in the environment.

In mammals the hormones are largely responsible for maintaining metabolism at a constant rate, for making the adjustments in absorption, anabolism, catabolism, excretion, et cetera, so that overall metabolism changes very little. Apparently, interrelationships among the hormones are responsible for this. For example, an increase in thyroxine in the blood speeds up the secretion of ACTH and the cortical steroids that tend to suppress thyroxine production. In turn, increasing levels of cortical steroids in the blood reduce the production of ACTH and the cortical steroids. In homotherms, the hormonal interrelationships are so closely associated with the nervous system that metabolism is finely balanced. The balance is such that a slight deviation in homeostatic function is immediately noted or evokes a response in an automatic corrective measure that is just sufficient in intensity to make the necessary adjustment. These same relationships probably exist in fish but have not reached as fine a balance, so that instead of a series of integrated checks and balances on metabolism such as those in the mammal the metabolism of the fish is controlled by a rather loosely integrated series of hormonal and neural relationships. With this type of control, hypo- or hyper-metabolic function would elicit a response by a corrective mechanism, but due to the lack of a sensitive regulating device, the corrective mechanism would over-correct. In this manner, hypothyroidism brought about by excess cortical steroid production would be followed by hyperthyroidism when the corrective relationship responded to subnormal thyroid function. This type of "teeter board" metabolism would be cyclic with respect to time.

The results of the determinations on the blood and serum of the smallmouth bass are characterized by systematic variation, a variation in which there is a trend. Such variations are apparent in 14 of the 16 components studied (see table 1). Possibly even more striking than the systematic variation noted has been the tendency for trends to reverse themselves. For example, the total serum protein levels decreased steadily for 8 weeks, then increased steadily for 12 weeks (fig. 2). These data also show an apparent relation among many of the components. Within the period August 2 to August 18, maximum or minimum values for 12 of the 16 components were recorded (figs. 2 to 12). The systematic variation, reversal of trends, and the tendency for these reversals to occur at about the same time in several components of the serum suggest that these changes are metabolic responses to a single physiological cause, and that this mechanism is actually responsible for the cyclic nature of the observations.

A hyperthyroid state was proposed and treated in detail in the section on Metabolism, page 27. Evidence in favor of increased thyroxine levels in the early part of the experimental period has been conclusive enough to warrant support of that idea. No attempt has been made to suggest the cause of this increase from June 24 to August 18 nor of its decrease after August 18.

Evidence has been previously presented that thyroid activity in fish increases in the spring of the year, and that this peak of activity lasts for only a few weeks (Swift, 1955; Barrington and Matty, 1954). Such cyclic activity almost certainly results from endocrine activity produced by the antagonistic effect of one hormone on another.

According to White et al. (1954), a number of gonadal steroids have been shown to affect the uptake of radioiodine, I^{131}, by the thyroid of the rat. Pickford and Atz (1957) reported the thyroid shows a lessening of activity during spawning. A period of decreased thyroid activity during spawning has been observed by Lieber (1936) in *Misgurnis fossilis*, a hibernating fish. Further evidence of the effect of spawning and sex hormones on the thyroid is presented by Hoar (1952), who noted that the mass mortality of the landlocked alewife following spawning may result from an exhausted thyroid. Summarizing extensive research on the effect of reproduction on thyroid activity, Hoar suggested that thyroid function during spawning may either be increased or diminished, depending on the life history and habits of the species (Brown, 1957).

The first blood sample in this study was taken on June 24. The time of the first sampling corresponded to the end of the spawning period of smallmouth bass in Fall Creek. At that time many abandoned nests were noted, and only a very few nests were still being guarded by the males.

It seems quite possible, then, that thyroid activity in the bass could be reduced during and immediately following the spawning period by the gonadal steroids acting as antithyroid compounds on the thyroid. Such a situation would reduce the amount of circulating thyroxine. Pickford and Atz (1957) have noted that the pituitary responds to low thyroxine levels by secreting additional amounts of thyrotropin, the thyroid activator hormone. Increased levels of thyrotropin would then lead to thyroid stimulation in the form of hypertrophy, hyperemia, and hyperplasia. All of these are conditions of hyperactivity in the thyroid; however, the synthesis of active thyroxine would still be inhibited by the sex steroids. Once spawning is completed and the level of circulating steroids has fallen (Pickford and Atz, 1957), the synthetic mechanism would be reconnected in the histologically hyperactive gland, resulting in hyperthyroidism. The resulting hyperthyroidism and its attendant reduction in energy production then could bring about the decreased anabolism and increased catabolism that have been proposed as causing a lowering of serum protein and lipid phosphorus levels, hypocholesterolemia, hypoglycemia, and as increasing the levels of sodium and potassium and nonprotein nitrogen in smallmouth bass between June 24 and August 18 of the experimental period.

No mention has been made thus far of possible causes for correction of the proposed hyperthyroid condition; however, the antagonistic effect of one hormone on another or hormonal relationships similar to those suggested as being responsible for the condition, were probably responsible for its correction. Pickford and Atz (1957), summarizing the work of several investigators, stated that the circulating level of thyroxine undoubtedly influences thyrotropin release by the pituitary. They further noted that treatment with thyroxine or thyroid preparation causes a decrease in thyroid function as reflected in the histological picture. With this evidence in mind, it seems probable that thyroxine synthesis has been inhibited here by increased thyroxine levels or that an increase in thyroxine resulted in a decrease in the release of thyrotropin, the precursor pituitary hormone. Because of a lack of thyrotropin to stimulate the synthetic and release activities of the thyroid, once the circulating thyroxine molecules were catabolized, there were few to replace them. With this reduced replacement rate, the high serum thyroxine levels began to fall in the bass between August 2 and August 18.

There is also a possibility that still another mechanism was brought into play to correct the hyperthyroid condition. Pickford and Atz (1957) state that administration of cortical hormones (these hormones are produced by the interrenal tissue in fish) causes an inhibition of thyroid function in the intact animal. White et al. (1954) also suggest that a high level of cortical steroids may have an inhibiting influence on thyroid function. Then, in addition to high levels of thyroxine being inhibitory in nature to thyroxine synthesis and release, an increase in blood levels of the cortical steroid hormones would bring about the same result—that of lowering thyroxine levels. This is even more plausible in the light of reports by Pickford and Atz (1957) and White et al. (1954) that administration of thyroxine will bring about an increase in production and release of the pituitary hormone, adrenal cortical trophic hormone (ACTH), which in turn promotes an increase in elaboration of the cortical steroids.

Further evidence that cortical steroids may have played a part in correction of the proposed hyperthyroid state has previously been presented in the discussion of blood glucose. It was suggested there that the biphasic increase in blood glucose between July 24 and August 27 (fig. 12) was a result of a biphasic release of cortical steroids, as proposed by Brodish and Long (1956).

From the evidence presented, it is suggested that the smallmouth bass has a twofold corrective mechanism for hyperthyroidism. Hyperthyroidism tends to be reduced by the high levels of thyroxine produced by the condition and also by increased synthesis of cortical steroids which was triggered by the same high levels of thyroxine.

Mention should be made here of the possible role of the thyroid in the quiescent state of the bass. Pickford and Atz (1957) have summarized several experiments by Olivereau (1954, 1955) noting the effect of low temperature on thyroid function in trout. In Olivereau's studies, low temperature enhanced the production of thyrotropin by the pituitary in the same manner in trout as in warm-blooded animals. This increased production of thyrotropin in turn produced the histological characteristics of hyperactivity in the thyroid; however, the fixation of radioiodine, I^{131}, into the thyroxine molecule was retarded. These experiments, summarized by Pickford and Atz, suggest that low temperatures may inhibit the synthesis of thyroxine. If this were correct, the bass in the present experiments would be expected to be in a state of hypothyroidism during the quiescent period; and, in fact, hypothyroidism could be the cause of the onset of the quiescent state. Hypothyroidism in mammals is best characerized by the word "slow." In hypohyroidism, the mammal is much less ctive, the pulse rate is low, the breathing ate is decreased, and excretion is rearded. These are also the characteristics f the quiescent smallmouth bass.

In conclusion, it should be stated that considerable amount of work needs to be lone on the phases of metabolism disussed in this paper, either to confirm or eject the hypotheses proposed. If furher work demonstrates the validity of the ypotheses proposed here, and if these hyotheses are applicable to all species of ish, then future fishery research must take hese proposals into account when designng experiments in which some phase of he metabolism of the fish is to be studied r is to be used to evaluate the effects of xperimental conditions. The results of he tests on the blood and serum of the mallmouth bass suggest that this fish, as n experimental animal, would not be comparable in any two given periods of time within a year. For example, the smallmouth bass would not be expected to react in a similar manner to the same set of experimental conditions on June 24 and August 18. If all fish have these cyclic metabolic patterns, then in an experiment to evaluate the growth response to a new diet, a low rate of growth would not necessarily mean that the diet is deficient; instead, a low rate of growth could be a result of a hyperthyroid condition. The response of fish in tests to determine vitamin requirements might be significantly different in two periods of the year even under identical, controlled experimental conditions. These different responses might not be a result of faulty experimental technique. The difficulty in duplicating the data in the two trials might also be the result of different vitamin requirements in different phases of the cyclic metabolic patterns.

SUMMARY

The concentrations of 16 noncellular omponents occurring in the blood and seum of smallmouth bass (*Micropterus olomieui*) were measured, including proeins, nonprotein nitrogen fractions, phoshorus fractions, electrolytes, and choleserol in the serum and glucose, creatinine, nd iron in the blood.

Blood samples were obtained from aproximately 350 fish obtained by angling rom a single stream. The blood was renoved directly from the heart with a yringe.

Measurements of concentrations of the ifferent components were made at aproximately 3-week intervals from June 4 to November 18, 1958, by colorimetric hemical methods.

Concentrations of 13 of the 16 compoents displayed systematic rather than random variation when plotted against time.

Concentrations of globulin, total phosphorus, and lipid phosphorus in serum decreased through successive samples until August. After August the concentrations of these components increased in successive samples through the end of the experimental period in November. Serum inorganic phosphorus concentrations decreased gradually throughout the experimental period, except for a sharp increase on August 18. Serum albumin concentrations remained relatively constant until globulin returned to its initial or June 24 value, after which both globulin and albumin increased in samples through the end of the experimental period.

Total nonprotein nitrogen and blood creatinine curves were almost exact duplicates with respect to shape. Concentrations of both fractions increased sharply then fell to their initial values by August 18. Concentrations of both components remained relatively constant through September, then increased in successive samples through the end of the experimental period in November.

Serum sodium and potassium levels tended to behave in an opposite manner to levels of the globulin and phosphorus fractions. The levels of these cations increased until August 18 and decreased afterward. Serum calcium concentrations, however, decreased in successive samples through August 27, then increased through the end of the experimental period.

Serum cholesterol levels remained constant in samples through August 2, dropped sharply on August 18, then increased to roughly two times the initial value by the end of the experimental period. Blood glucose concentrations, in general, increased throughout the experimental period. Serum uric acid, serum chloride, and blood iron concentrations behaved in somewhat cyclic manner, but not to the extent of the components previously discussed.

Blood samples taken on August 18 showed highest concentrations reached by uric acid, inorganic phosphorus, sodium, and potassium, and lowest concentration of nonprotein nitrogen, creatinine, tota protein, globulin, and cholesterol.

Available data suggest that changes i the anabolic-catabolic balance were responsible for the cyclic concentrations o the various components and that the mos likely explanation of these changes i in the changes in levels of circulatin thyroxine.

Antagonistic effects of the sex hormone during spawning and of circulating thyroxine on the thyroid gland are suggeste as possible causes for the changes in level of circulating thyroxine.

It is suggested that the antagonistic effect of one hormone on the production o another hormone produces a total metabolism in the smallmouth bass that would b cyclic in nature when plotted against time

If metabolism in fish is cyclic with respect to time, the norm can be determine only if the time of year the blood sample were collected is included in the data This suggests that in experiments in whic some phase of metabolism is used to measure effects of a treatment the results (response of the fish) are dependent on tim of year the experiment is performed Such results would be applicable onl when the metabolic rates of the experimental fish and of the fish from which th data came are of the same magnitude.

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CHRONIC EFFECTS OF ENDRIN ON BLUNTNOSE MINNOWS AND GUPPIES

Donald Irvin Mount

RESEARCH REPORT 58

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Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*

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Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE
Daniel H. Janzen, *Director*

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ABSTRACT

In an investigation of chronic toxicity of endrin to bluntnose minnows and guppies, conducted for 291 days, neither species could withstand concentrations greater than 0.5 parts per billion of endrin in water for more than a few days. Less than 50 percent of the test animals could live in 0.5 p.p.b. for more than 30 days. Approximately 65 percent could live in 0.4 p.p.b. for periods exceeding 30 days, while there was little mortality due to endrin in 0.25 and 0.1 p.p.b. endrin in water.

Fish suffering from endrin poisoning exhibited symptoms (increased ventilation rate, convulsions, and loss of equilibrium) which indicate that it affects the central nervous system. Sublethal concentration caused increased activity and hypersensitivity to stimuli. No cell damage was found in fish which were able to survive in endrin concentrations, and essentially no physical effects other than increased activity were noted. Adult female guppies ceased to have young when placed in endrin concentrations, but the gonads of the bluntnose minnows were approaching sexual maturity when the test was terminated.

In continuous-flow acute-toxicity tests, it was found that the 96-hour TL_m values ranged from 0.27 p.p.b., for fish 30 mm. standard length, to 0.47 p.p.b., for fish 60 mm. standard length.

Endrin had no effect on oxygen consumption in the seven bluntnose minnows tested. Neither did acute exposure to endrin significantly affect the ability of the fish to swim against a current of water.

In carp exposed to concentrations of 2.5 to 10 p.p.b. endrin during periods varying from 2.5 to 28 days, the digestive tract, liver, heart-spleen-blood, and kidney contained the highest accumulations of endrin. The maximum concentrations of endrin found in the tissues were approximately 160 times greater than that in the water in which the fish were living. The concentration of endrin in the heart-spleen of a carp exposed 28 days to 2.5 p.p.b. was found to be 400 p.p.b. Muscle tissue was consistently low in endrin content. The gills were always low or negative in endrin content, whereas the digestive tract was consistently high, indicating that endrin probably enters the body through the intestine.

Endrin does not appear to be cumulative in its effects on guppies and on bluntnose minnows. Little or no tissue damage was found in fish which could live in water containing endrin, and fish seemed to recover completely from one exposure to endrin. The increased activity caused by very low concentrations of endrin could be very damaging to fishes in natural waters, disrupting spawning and subjecting the fish to predation and other decimating factors.

HRONIC EFFECTS OF ENDRIN
N BLUNTNOSE MINNOWS AND GUPPIES

y **Donald Irvin Mount**

hio State University

In the past 15 years, the use of pesticides as increased rapidly, and the number of ompounds used for pest control has grown t an astounding rate. Abstracting jourals in recent years have had to add a subtle for articles about pesticides, and each ear this section becomes larger. Herbiides have increased in use in a similar anner, and at present are replacing culivation in the production of some agriculural crops (corn, for instance). On the ommercial market, one finds a whole aray of compounds for combating roaches, nts, spiders, mites, and other pests. ities now have spraying programs in hich every alley is sprayed for fly and osquito control. Highway departments pray roadsides with herbicides instead f mowing. The Federal and State govrnments, often in cooperation with local overnments or agencies, spray large areas or various insect pests. The use of hemicals, especially DDT, for the conrol of forest pests is of ever-increasing mportance.

Recently a huge aerial-spraying proram has been conducted in the southeastrn United States in an attempt to control he imported fire ant. Various extermiating agencies now use new chemicals to ontrol termite damage to buildings, and ften give a guaranteed protection period which may last several years. Marine paints on the market contain chemicals to ontrol arthropod damage to wooden boats; floor waxes contain pesticides for ant and roach control. Some insecticides have been found very effective in rodent control, especially in orchards. Many conservation departments use chemicals to eradicate fish populations in large bodies of water, and it appears at present that effective control of the sea lamprey in the Great Lakes may be achieved by use of chemicals. In short, one will find pesticides used in almost any place he may go.

Such widespread use has caused many people to wonder what effect these chemicals may have on the intricate balance of animal populations. Unfortunately, many of the pesticides used are not very specific in their effects. These pesticides may alter food chains by removing a certain group of animals. A long residual life as well as high toxicity are sought-after features of pesticides, and for extended periods of time after spraying they are dangerous to animals other than those being controlled.

Aquatic animals in particular are highly susceptible to pesticides. As an example, Fitzwater (1958) reports that in 1953 an airplane carrying a spray mixture containing a little over 1 gallon of actual endrin crashed into a Louisiana bayou. This resulted in the death of all fishes and reptiles, including 42 alligators, for a distance of 35 miles from the point of the crash. Aquatic animals are often exposed to insecticides as a result of runoff from treated areas, as well as from direct application to the water.

There is a dearth of information on the residual life of insecticides in soils and in organisms. Young and Nicholson (1951) reported what they thought to be the first recorded large-scale kill of aquatic animals caused by agricultural use of insecticides. There were 15 major fish kills in northern Alabama in one summer, and in Flint Creek in Alabama, in a 13-mile section varying from 6 to 20 feet in depth, there was an apparent total eradication of the fish population. These kills were thought to be due to frequent applications of insecticides, followed by several heavy rains in August which washed the insecticides from the soil into the streams. Barker (1958) found robins in Illinois dying from feeding on earthworms after heavy rains in the spring. Analysis of the worms showed high concentrations of DDT, which had been sprayed on the area almost 1 year earlier. Barker found the DDT content of the soil to increase in the autumn, presumably owing to the residue on the falling leaves. Barlow and Hadaway (1955) found DDT recoverable from soil 12 months after sorption was complete. Harrington and Bidlingmayer (1958) found extensive kills of aquatic animals resulting from aerial applications of 1 pound of dieldrin pellets per acre in a tidal marsh. They noted crabs eating moribund fishes and dying themselves the next day.

Hunt and Bischoff (1960) have presented a very complete picture of the accumulation of DDD (dichloro-diphenyl-dichloroethane) in animal tissues. In this case, Clear Lake in California was treated three times with DDD. The first treatment was applied in 1949 at a concentration of 1 part in 70 million, the second in 1951 at 1 part in 50 million, and the third in 1957 at the same concentration as the second. They found concentrations a high as 2,275 parts per million in the fa of apparently healthy largemouth bas and concentrations as high as 42 parts pe million in the fat of yearling largemout bass hatched 7 to 9 months after the las application. Carnivorous fish had highe levels of DDD in their tissue than di plankton feeders.

All these investigations and many mor not cited point up the need for cautiou use of insecticides, and for extensiv studies to determine what the total effect o their use may be over a long period.

Certainly in coming years, as the worl population increases, the value of our inland waters for recreation as well as foo supply will be increasingly important, an the aquatic habitat must be protected. A the present increased rate of use of pesticides, it is not impossible that even th oceans along the continental coasts ma receive enough to reach a toxic level. Th unfortunate fact is that in general ther is little concern about the danger until desirable organisms begin to die, and ofte it is then too late to remedy the situation

Acute testing of new chemicals on various animals is rapidly becoming standar procedure before the chemicals are released to the public. Toxicity is usuall measured by the number of deaths, but sublethal effects also can be very damaging The need for chronic-toxicity studies is becoming more pressing and is gaining som support. The importance of findin symptoms of poisoning at a time whe complete recovery is still possible is slowl being recognized. In an effort to focu attention on this area, as well as to determine some of the effects of subletha doses, an investigation of the chronic toxicity of endrin to fishes was initiated. Endrin was chosen because of its extrem toxicity to fishes and its increasing use a a pesticide. Henderson, Pickering, an Tarzwell (1959) state, "Endrin was by fa he most toxic of the insecticides to all species of fish [tested]. In fact, it is the most toxic chemical that has been tested n this laboratory." They found endrin o be lethal in 96 hours to 50 percent of the bluegills tested at a concentration of 0.6 p.p.b. (parts per billion) in the water.

Endrin is 1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*endo*-5-8-dimethanonaphthalene. It is a yellowish-white crystal with a sweet odor when it is in a dry form. Endrin is slightly soluble in water and moderately soluble in alcohol.

The use of endrin has increased each year since it was first introduced in the early 1950's. Officials of one agricultural chemical company informed me that they sold several thousand pounds of active endrin in a 3-State area during 1958. Endrin has been used effectively to control many pests. A few are listed below to illustrate its broad spectrum of toxicity.

Other pests such as army cutworms, plant-lice, tent caterpillars, and white-pine weevils have been effectively controlled.

Although such application rates may seem low, Tarzwell and Henderson (1956) working with a closely related compound, dieldrin, found runoff from the first and third rainfalls from an area treated with 4.66 pounds of dieldrin per acre to be lethal to 50 percent of the bioassay fish in 96 hours. Runoff from the fourth rain still contained some dieldrin, as indicated by the reactions of fish placed in a sample of it. Rosen and Middleton (1959), by use of a carbon filter, found 0.1 and 0.013 p.p.m. in the runoffs from the first and third rains in the treated area mentioned above. These two experiments, together with many published accounts of fish mortality, indicate that these insecticides do reach the streams in sufficient quantity to be toxic. Mr. Henderson has informed me that "quite constant amounts" of insecticides are found in some streams. Undoubtedly the concentrations increase in periods of widespread spraying (April and May), but it is significant that there is a measurable level of insecticides in the streams at all times. Presumably these levels are maintained by residues of insecticides in the soil which have been shown to last for at least 1 year (Barlow and Hadaway, 1955). Seasonal spraying renews this supply in the soil. This fact makes the need for studies of chronic effects even more pressing.

A literature search revealed no studies on the chronic effects of endrin on fishes, although some experiments have been conducted on birds and mammals. Treon, Cleveland, and Cappel (1955) found that all rats fatally poisoned by endrin had "diffuse degenerative changes in the liver, kidney, and brain." In a rabbit given 50 oral doses (1 mg./kl.), endrin caused "diffuse degeneration and fatty vacuolation of hepatic and renal cells and degeneration of the heart." Survival times of male rats fed 100 and 50 p.p.m. endrin in their food, and of females fed 100, 50, and 25 p.p.m., for periods up to 106 weeks were significantly shorter than for those animals fed 1 and 5 p.p.m. Concentrations of 50 and 100 p.p.m. in food caused the ratio between liver weight and body weight to be larger

USE OF ENDRIN TO CONTROL CROP PESTS

Pest	Crop	Dosage
Spiny bollworn	Cotton	500 grams per hectare.
Lesser peach borer	Peaches	1 qt. emulsified endrin per 100 gal. (0.5 gal. per tree.)
Potato tuber moth	Potatoes	0.25-percent spray.
Red-banded thrips		1 pt. per 100 gal.
Potato leafhopper	Potatoes	2 cc. per gallon of 19.5-percent endrin.
Citrus psylla	Citrus fruit	0.01-percent endrin.
Pine mice	Orchards	2.5 lbs. per 52 trees.

than normal. Male rats fed 5 and 25 p.p.m. for 2 years also showed an increased liver weight to body weight ratio.

They also stated that, in general, the only effect in female dogs fed 4, 3, and 1 p.p.m. for 6 to 19 months was some slight renal tubular vacuolation. Some dogs fed 3 p.p.m. for 19 months had enlarged kidneys and hearts. Those dogs which survived on 8 p.p.m. had normal viscera, except for slight diffuse degeneration of the distal convoluted tubules in the kidneys.

DeWitt (1956) reported that 10 p.p.m. endrin fed to adult pheasants during the reproductive period reduced hatchability of eggs and survival of the young. One p.p.m. fed to quail before the reproductive season, but discontinued during reproduction, reduced the fertility and number of eggs, as well as the survival of the young.

Sherman and Rosenberg (1953) found 3.5 mg./kg. of endrin in 7-day chickens to be lethal between 6 hours and 5 days. It caused degeneration of the liver and kidney, and following long exposures was stored in peritoneal fat and the liver.

Kunze and Laug (1953) detected storage of endrin in the brain and liver of rats. Females contained more fat-stored endrin than males. These investigators recovered considerable amounts of endrin from the kidneys, but this was of a "different chemical nature."

Ely, Moore, Carter, and App (1957) reported that 20 mg. or more per day fed to cattle caused measurable amounts of endrin to occur in the milk. One and one-half milligrams per kilogram per day caused the cattle to exhibit toxic symptoms.

Data on the effect of endrin on fish are very scanty. Henderson, Pickering, and Tarzwell (1959) state that in soft water (alkalinity 18 p.p.m., hardness 20 p.p.m.) at 25°C. the 96-hour TL_m (median tolerance limit) was 0.6 p.p.b. for bluegills, *Lepomis macrochirus*, 1 p.p.b. for fathead minnows, *Pimephales promelas*, and 1.9 p.p.b. for goldfish, *Carassius auratus*. These fishes were 1.5 to 2.5 inches long. Guppies, *Lebistes reticulatus*, 0.75 to 1 inch long, had a 96-hour TL_m value of 1.5 p.p.b. under the above-mentioned conditions. These were static tests with five guppies in 2 liters of test solution and five fishes in 10 liters of solution for the other three species. In constant-flow tests of 20 days' duration, they found endrin to be toxic at levels of half the 96-hour TL_m value. Their conclusion was that endrin had a cumulative or chronic effect on fishes. They concluded also that natural variations in pH, alkalinity, and hardness have no major effect on the toxicity of the chlorinated hydrocarbon insecticides to fishes, although endrin, among others, was slightly more toxic in soft water.

Iyatomi et al. (1958) determined the 48-hour TL_m for 5- to 7-centimeter carp to range from 140 p.p.b. at 7°–8° C. to 5 p.p.b. at 27°–28° C. They found that the 24-hour TL_m for day-old carp was 8,500 p.p.b., which decreased to 46 p.p.b. at 6 days of age. It is quite probable that these TL_m values are high, since five fishes (5 to 7 centimeters long) were placed in 2 liters of water. Such large fish in 2 liters of water would absorb endrin and thereby reduce the concentration.

Both of the last two papers mentioned considered the effect of the form in which the endrin was administered (powdered, granular, or emulsion) on the lethal dose. Form was not considered in the current investigation. Form probably affects the amount dissolved in the water and not the actual toxicity.

Objectives

The objectives of this investigation were—

1. To determine the maximum concentration in which guppies and bluntnose

minnows could survive for extended periods.

2. To determine reproductive and physiological effects of sublethal concentrations of endrin on fishes.

3. To determine the place of entry into, and the movement through, the fish's body.

4. To determine any differences in the acute toxicity of endrin to various size groups.

5. To determine whether there is a potential danger to human beings or to other animals which eat fishes that have been exposed to sublethal doses of endrin.

The methods and equipment employed in this investigation are described separately with each phase of the investigation. Raw data are given in the appendix. As used here, the terms "chronic test" and "acute test" refer to tests of long and short duration, respectively.

This report was prepared in partial fulfillment of the requirements for the degree of doctor of philosophy in the Graduate School of Ohio State University. Dr. Wilson Britt was my major advisor and Dr. Eugene Dustman was my coadvisor during my doctoral study. In addition, I am indebted to Drs. William Spoor, David F. Miller, George Ware, Frank Fisk, and William McIntosh for advice and assistance.

CHRONIC-TOXICITY TESTS ON BLUNTNOSE MINNOWS

Objective

The objective of this experiment was to determine the effects of sublethal levels of endrin on bluntnose minnows exposed for extended periods of time.

Methods and equipment

The chronic-toxicity testing was based on a continuous flow system. The apparatus consisted of a reservoir capable of holding a 4-day supply of the test solution, a constant-head chamber, and a test chamber, all shown in figures 1 and 2.

The reservoirs were made of ¾-inch marine plywood coated with four coats of plastic resin in which glass cloth was imbedded for strength (Mount, 1959). Ten such reservoirs, 16 by 16 by 24 inches, were arranged in two batteries of five each. They were supported 4 feet above the floor by six 4-by-4 legs and eight 2-by-6 planks. Glass tubing was bent as shown in figure 2 to serve as water-level gauges.

Water was siphoned from the reservoirs into a constant-level chamber. Figure 3 shows the details of the float valve used to control the water flow into the constant-level chamber; the level did not vary by more than ¼ inch during operation. Automotive valve-grinding compound was used to seat the ground-glass surfaces. No sealing compound was used on the valve seats; only a slow seepage occurred when the valve was closed. Large cork stoppers coated with plastic resin served as floats. Ten of these valves functioned for periods of 6 to 11 months with approximately 10 failures due to particles of dirt.

Water was siphoned from the constant-level chamber into the test chamber, and the flow was controlled by small glass jets. These jets were made by heating and drawing glass tubing to a very small diameter. The jet was fastened to the water line, and small pieces were broken off until the desired flow rate was obtained. Glass stopcocks and screw clamps were tried, but both failed to maintain a constant water flow and became clogged quickly with dust particles. Flow rates were measured by a 10-milliliter graduated cylinder. All water lines were made of glass tubing with tygon tubing at the joints.

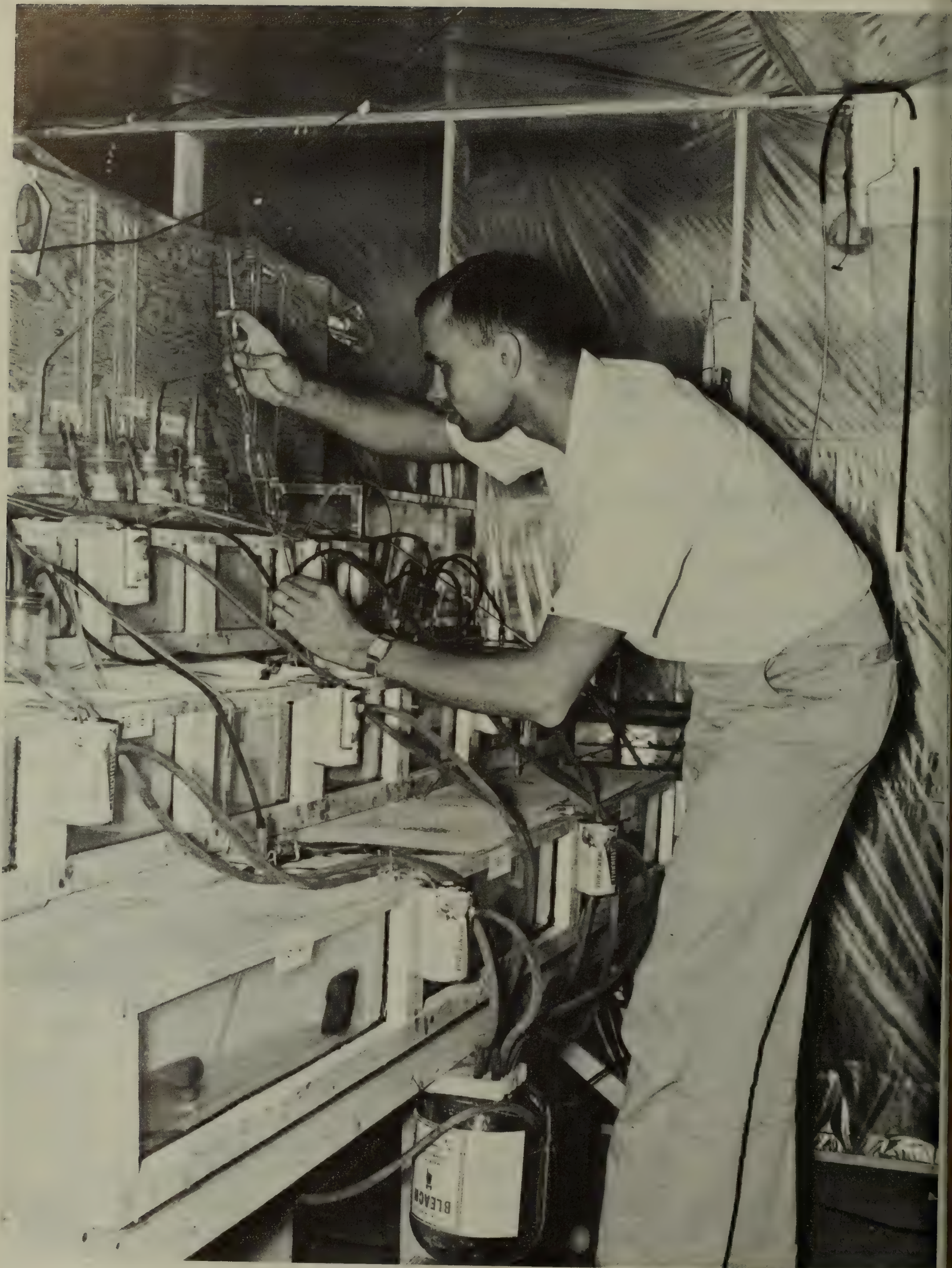

FIGURE 1.—Continuous-flow system used for the chronic-toxicity testing.

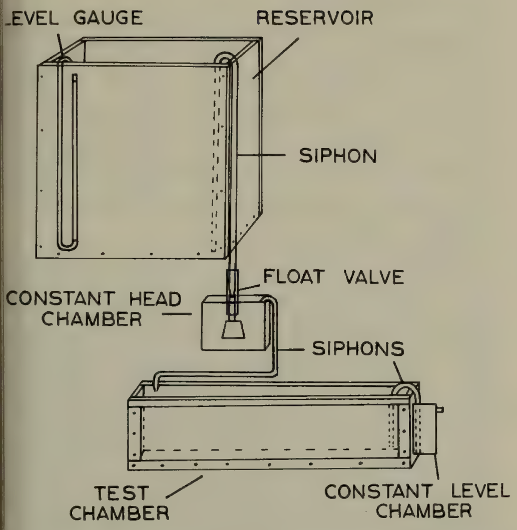

FIGURE 2.—Diagram of continuous-flow system used for chronic-toxicity testing.

The bluntnose-minnow test chambers, 10 by 24 by 6 inches, were constructed in the same way as the reservoirs, except that the front was glass, and white pigment was added to the plastic resin. A narrow retaining strip was placed around the front to hold the glass in place. Double-strength window glass was cut so that it was as large as the entire front, and was sealed in place with aquarium cement. A strip of aquarium cement ⅛ inch wide or less, around the glass, was exposed to the test solution. Each test chamber, when filled to normal level, had a capacity of approximately 18 liters. A U-shaped glass tube siphoned the water from the test chamber into a constant-level chamber, and from there it was emptied into the drain. The end of the tube in the test chamber was inserted into a cylinder made of plastic screen in order to prevent fish from entering the siphon tube. The test chambers were joined in batteries of three to seven, and arranged in three banks or levels, all lower than the reservoirs. Compressed air was bubbled into the water in each chamber through a glass jet.

Since the endrin was added directly to the reservoir, the level gauge of each reservoir was calibrated so that exactly 80 liters of water could be measured into it. In this way the desired concentration could be made directly in the reservoir. There were 2 inches of water under the zero level mark in the level gauge, so that when the reservoir was lowered 80 liters the columns of water in the level gauge and siphon tube were not broken. A change in volume of 0.1 percent could be detected on the level gauge. Each reservoir held a 4.5-day supply of solution which flowed out at the rate of 720 ml. per hour. This rate amounted to the approximate capacity of the test chamber in 24 hours.

In 1.5 hours, all 10 reservoirs could be drained and refilled with fresh solutions. During the filling, the siphon tubes were clamped to prevent an improper concentration from reaching the test chambers.

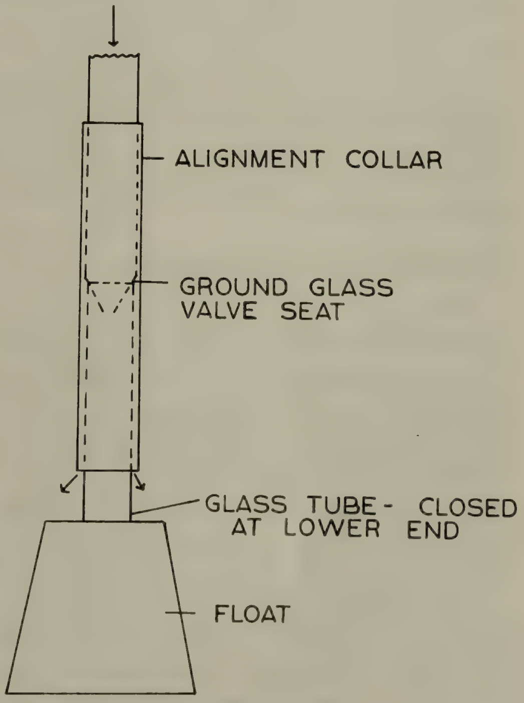

FIGURE 3.—Float-valve assembly for regulating water-flow.

This entire unit was set up in a basement room built especially for the experiment. The walls were made of two sheets of polyethylene-plastic film with 4 inches of dead-air space between them. Sunlight from a basement window entered through the plastic wall; thermostatically controlled electric heaters held the temperature constant. It was found that small variations in air temperature did not alter water temperature measurably. There was temperature stratification of the air; but this proved to be advantageous because the water in the reservoirs was 1° to 2° C. warmer than that in the test chambers. Influent was introduced at the water surface in the test chamber, and since it was less dense it spread out over the surface and settled to the bottom as it cooled. The outlet siphon drew the water from the bottom. This movement, plus the turbulence from aeration, gave good water circulation through the test chamber as determined by methylene-blue dye. Four test chambers and four reservoirs were used for the bluntnose-minnow tests. Three test chambers contained endrin and were designated TB–6, TB–7, and TB–8; one designated CB–10, without endrin, served as a control.

Physical and chemical conditions

The chronic-toxicity tests were conducted at a constant temperature of 22.2° C., plus or minus 1° C. The summer of 1959 was unusually warm, and on several days the capacity of the cooling unit was exceeded. For 3 days in August, the temperature reached 25° C. in the late afternoon, and for a total of 18 days in the months of June, July, and August the temperature exceeded 23.2° C. but did not reach 25° C. I feel that this variation did not affect the results significantly, since this was a chronic test, and because any variation due to temperature fluctuation would affect the control group as well as the test fishes. During all months exce June, July, and August the temperatu rarely varied by more than plus or mini 0.5° C. Continuous temperature recor were kept by a recording thermograp Examination of these records showed th no large temperature fluctuations occurre at any time.

Water chemistry in the test chambe was determined periodically, and the p and alkalinity of the tap water were mea ured each time before the reservoirs we filled. Chemical analyses of the tap wat upon leaving the Morse Road water-trea ment plant are shown in table 1. This i formation was made available through t courtesy of Mr. Richard Melick, Divisio of Water, Columbus, Ohio. An analys by the State health department on N vember 19, 1959, showed, in additio fluorides 0.2 p.p.m., silica 4 p.p.m., nitra nitrogen 0.5 p.p.m., and sodium 7 p.p.m.

TABLE 1.—*Chemical analyses of Columbus t water*

[All values except pH are expressed in p.p.m. These val were determined by the Columbus Division of Water on t water as it left the Morse Road treatment plant]

	Oct. 21, 1959	Nov. 25, 1959	Dec. 28, 1959	Feb. 196
Total alkalinity	22	22	20	21
Phenolphthalein alkalinity	4	3	12	1
Noncarbonate hardness	52	54	62	71
Total hardness	74	76	82	9
Calcium	18	21	24	2
Magnesium	7.5	6	5	
Iron	0	0	0	
Chlorides	7.5	8	7.5	
Sulphates	52	58	62	6
Free CO_2	0	0	0	
pH value	10.1	8.5	10.2	1
Turbidity	.2	.25	0	
Total solids	153	139	148	16
Manganese	.3	0	0	

According to the analysis made at m laboratory before the reservoirs we filled, the chemical characteristics of t tap water used in the tests, based on 7 samples over a period from April 11, 195 to January 27, 1960, were as follows:

	Range	*Mean*
pH	6.5– 8.5 p.p.m.	7.8 p.p.
Total alkalinity	15.0–42.0 p.p.m.	23.9 p.p.

Only two alkalinity determinations exeded 30 p.p.m., and only two pH readgs were below 7.0. Phenolphthalein allinity was always present in tap water, ut disappeared during aeration as the servoirs were filled. Free chlorine apeared in the water for the first time on ovember 29, 1959, and filtering through arcoal was necessary from then until the d of the test. The filter consisted of a foot length of glass tubing, 100 mm. inde diameter, packed with granular bone arcoal. Chlorine determinations were ade according to the starch-iodine test escribed in Standard Methods for the Exmination of Water and Sewage (1949).

Table 2 summarizes the chemical charteristics of the solutions in the test chamers. No phenolphthalein alkalinity was und in the test chambers. The unmodied Winkler method was used for dislved-oxygen determinations, and issolved carbon dioxide and alkalinity ere determined by standard methods escribed by Welch (1948). Indicator lutions with a color chart were used to etermine pH. A comparison of this ethod with a pH meter indicated that the ethod was sensitive to 0.25 pH units.

The chronic testing was begun on pril 11, 1959, and was conducted for 291 ays, ending on January 27, 1960. Five luntnose minnows, *Pimephales notatus*, ere placed in each of the four test chamers. They were assigned to chambers at andom by the use of a table of random ligits. Rocks of similar composition vere placed in each chamber to serve as over. Hardware cloth of ¼-inch mesh vas placed over the top to keep the fish rom jumping out. Ground commercial log-chow checkers were free-fed twice laily. This diet was supplemented from ime to time with chopped earthworms taken from an area known to be free from insecticides. The fish were overfed intentionally, so that all fish were able to eat as much as they would. The excess food and excrement were siphoned daily.

This species fights very little under laboratory conditions, and its behavior, plus its tolerance to laboratory conditions, makes it a very desirable test animal. All fish used in the chronic test were collected in a small pool in Rocky Fork Creek, on the Franklin-Delaware County line, on March 16, 1959. The fish were held in the laboratory at 22° C. for 26 days before the test for chronic effects was begun. Those not used immediately in the tests were held at 22° C. for later use.

Table 2.—*Chemical analyses of water in the test chambers based on determinations made approximately every 9 days during the 291-day test period*

[All values except pH are expressed in p.p.m.]

Chemical character	Range	Mean
Total alkalinity	18–33 (One reading over 28)	24.0
Dissolved oxygen	5.3–7.2	6.2
Dissolved CO_2	1–3	2.3
pH	7.0–7.8	7.4

Ninety-six percent pure reference standard endrin was used in the tests. A stock solution of 1 mg. endrin per milliliter of 95-percent ethyl alcohol was prepared, and the desired amounts were measured with a 1–milliliter-graduated pipette. Initially, 200 ml. of the stock solution were prepared. After this had been used, one liter of solution was made and this was sufficient for the rest of the chronic test and for all of the acute testing.

When filling the reservoirs, I dropped the appropriate amount of the stock solution into a beaker containing approximately 100 ml. of water. The beaker was then emptied into the reservoir and washed for several minutes under the water stream entering the reservoir. It was necessary to keep the alcohol content as low as possible, because it permitted a considerable growth of bacteria, even in low concentrations. A volume of pure

95-percent ethyl alcohol, equal to the largest volume of alcohol put into the test chamber of highest endrin concentration, was put in the control chamber. This originally amounted to 0.72 ml. per 80 liters of water, but later when the maximum test concentration was dropped to 0.5 p.p.b. endrin, it was decreased to 0.4 ml. per 80 liters of water. Even this amount permitted an appreciably greater bacterial growth than did the 0.08 ml. put into TB–8. The stock solution was measured near the floor to avoid splashing endrin into the test chambers. The bottle containing the stock solution, and the pipette, were frequently wiped with a cloth saturated with pure 95-percent alcohol to remove any traces of endrin residue.

During the testing period the need for determining the concentration of endrin in the test chambers became apparent. The best chemical test for endrin is sensitive only to 5 p.p.b., but by bioassay it was possible to determine concentrations as low as 0.4 p.p.b. In November 1959, during acute-toxicity testing, it was found that 100 percent of the fish 20 to 40 mm. standard length would die in concentrations as low as .4 p.p.b. in less than 4 days. Fish of similar sensitivity were used to determine the concentrations in the test chambers by bioassay techniques. In acute tests in which the concentration was better known, fish died in 1 to 2 days at 0.6 p.p.b. and in 2 to 3 days at 0.4 p.p.b.

A 5-gallon glass jug with the top cut off was placed so that the effluent from TB–7 flowed through it. Later, this was also done with TB–6 and with the influent to each of these chambers. The results are summarized in table 3. By comparison with known concentrations, these data show that the concentration in TB–7 was approximately 0.5 p.p.b., while the concentration in TB–6 seems to have been somewhat lower than 0.4 p.p.b. Since these concentrations are very near the threshold of tolerance, the fact that no fish died in the TB–6 effluent may not be significant. After the chronic test was ended, five fish ranging from 29 to 38 mm. standard length were placed directly into TB–7, and all died within 3 days. Five others were placed in TB–6. One disappeared, but the other four died within 3 days, indicating that the concentrations were as expected. (Despite precautions, fish were occasionally lost by jumping out onto the floor.) Although the actual concentrations may have been slightly lower than the calculated concentrations in some chambers, this does not introduce serious errors because the concentrations in TB–6 and TB–7 were the maximum that the fish could withstand.

The small loss of endrin may have been a result of two factors: absorption by the fish and loss because of aeration. The manufacturers both of the plastic resin used to coat the reservoirs and chambers, and of the endrin, gave assurance that there would be no reaction between the endrin and the plastic. The concentration was changed in several chambers and, so long as they were washed thoroughly with soap and water, there was no residual insecticide found in the water.

Table 3.—*Bioassay of test concentrations of the chronic-toxicity tests*

[Young bluntnose minnows were used as bioassay animals. Survival rates in known concentrations are taken from acute-testing results. See appendix table 1]

Source of water	Number of fish	Size range, mm. S.L.	Average survival in days
Effluent from TB–7	5	25–30	2.2
Do	4	54–59	4.0
Influent to TB–7	5	22–33	2.4
Effluent from TB–6	5	25–30	(1)
Influent to TB–6	3	29–35	5.3
Known concentration, .6 p.p.b.	5	29–34	1.0
Do	5	26–31	2.0
Known concentration, .4 p.p.b.	5	25–34	2.4
Do	5	26–32	3.0

[1] No deaths.

In order to determine the effect of aeration on the loss of endrin from water, 9 ters of a 5-p.p.b. solution were made and ivided equally into two battery jars. ne was aerated vigorously with five air ets for 72 hours; the other solution was ot aerated. At the end of 72 hours, five ish were put into each jar. All fish died n 12 hours in the unaerated solution, but one died within 48 hours in the aerated ne. This evidence indicates that aeraion does remove at least some endrin. eration was very slow in the test chamers and previously cited evidence indiates that there was a minimal loss.

esults

Initial test concentrations were selected n the basis of 48-hour screening tests. oncentrations of 0.9, 0.5 and 0.1 p.p.b. were used for chronic testing. The survival times are listed in table 4. As fish died in the various chambers, other fish were fin-clipped for identification and were placed in the chambers to maintain five animals per chamber.

A summary of the growth data on fish living for extended periods is given in table 5. Means are expressed as individual growth per fish per day. The mean for each group was calculated as follows:

$$\frac{G}{T}=\text{mean daily growth.}$$

T=Total number of days that n fish lived.

G=Total growth of n fish.

n=Number of fish in the group.

Discussion

Fish poisoned by endrin exhibited symptoms clearly indicative of central-nervous-system disorders. The first indication of poisoning was an increase in ventilation rate accompanied by rapid, jerky movements of the body and fins. Almost simultaneously, an increased sensitivity to external stimuli would be noticeable. Next, the fish would move to the surface, and shortly thereafter begin turning

TABLE 4.—*Summary of the chronic-toxicity tests on bluntnose minnows*

Concentration of endrin	Chamber	Sex	Initial size (mm. S.L.)	Number of days lived	Growth (mm.)
0.9 p.p.b.	TB-6	F	46	9	----------
		F	36	11	----------
		M	42	6	----------
		------	42	3	----------
		------	36	5	----------
		------	36	4	----------
0.75 p.p.b.	TB-6	F	41	9	----------
		M	36	9	----------
		M	37	10	----------
		M	38	29	----------
		------	38	9	----------
0.5 p.p.b.	TB-7	F	50	81	----------
		F	36	38	----------
		F	49	[1]290	8
		F	35	[1]291	23
		F	56	7	----------
		F	57	11	----------
		F	60	4	----------
		F	45	7	----------
		F	48	[1]106	3
		M	43	17	----------
		M	51	[1]106	5
		M	45	[1]105	5
		------	33	11	----------
		------	38	6	----------
0.4 p.p.b.	TB-6	F	59	[1]194	0
		F	39	196	13
		F	45	10	----------
		F	39	[1]107	3
		M	53	[1]231	19
		M	47	[1]196	8
		------	55	11	----------
		------	37	8	----------
		------	36	10	----------
		------	36	12	----------
0.1 p.p.b.	TB-8	M	50	247	21
		M	49	[1]291	23
		M	37	[1]291	32
		M	35	[1]291	33
		M	31	[1]291	35
0 p.p.b.	CB-10	F	45	274	14
		F	35	269	23
		F	36	[1]291	19
		M	69	[1]51	5
		M	47	[1]291	20
		------	39	[2]62	----------

[1] Killed at the end of the test.
[2] Jumped out of test chamber.

TABLE 5.—*Mean daily growth of bluntnose minnows*

[In millimeters. Number in parentheses is number of fish on which the mean is based]

Concentration of endrin	Chamber	Mean daily growth of—		
		Males	Females	Both sexes
0.5 p.p.b.	TB-7	0.047 (2)	0.050 (3)	0.049 (5)
0.4 p.p.b.	TB-6	.063 (2)	.032 (3)	.047 (5)
0.1 p.p.b.	TB-8	.102 (5)	---------- ----------	.102 (5)
0 p.p.b.	CB-10	.073 (2)	.067 (3)	.069 (5)

around and around rather slowly, frequently swimming backwards. Convulsions would follow, and between them the fish would vibrate. The fins and body were not still for a second, and the fish jumped out of the water frequently. Convulsions would become more severe and of longer duration; finally, loss of equilibrium would occur. The fish often lived several hours after loss of equilibrium, and would make periodic darts around the chamber, often "barrel rolling" in the process. Breathing would then stop, but usually the heart would continue to beat for 10 to 15 minutes. The body would be limp at this time, but as rigor mortis set in, a characteristic bend in the spine would occur at about the region of the anus, so that the body formed an obtuse angle. During the period of poisoning, there was a progressive darkening of body coloration until the fish died.

In weaker concentrations, the above symptoms were prolonged over several weeks. Some fish were in the stage of hyperactivity for many days. The two females that lived 290 and 291 days in 0.5 p.p.b. (table 4) were in such a state for 2 months. They became emaciated; their abdomens caved in so that they appeared humpbacked. (This may have been an illusion due to the hollow abdomens.) When food was dropped into the chamber, they attempted to feed, but usually they were unable to grasp the food, often missing it completely, as if their coordination were impaired. For a period of several weeks they ate little if anything. On June 10, 1959, two fish were put into the chamber with them. The latter two died within 11 days, but the original two females began to feed, and by July 1, had begun to gain weight and appeared normal. The behavior of these females during the first 2 months was typical of many other fish which had a prolonged period of poisoning before death.

Because of the limited facilities, only a very small number of fish could be used in the test; therefore, any conclusions drawn from the data must be made with caution. Small numbers were employed to avoid crowding with the hope that under these conditions the fish would spawn.

The high growth rates of those fish in 0.1 p.p.b. stand out as the most significant difference in table 5. This growth was greater than that of the controls, and began very early in the test period. Fish were not measured during the test period because of the danger of injury. Unfortunately, all the fish in 0.1 p.p.b. were males, and the higher growth rate may be due to this fact. It is true, however, that the females grew more than the males in one out of three chambers in which both sexes were present. This is an indication that the larger length-increase in 0.1 p.p.b. endrin was not attributable to the fact that all were males. One might postulate many reasons for this growth rate, but the significant point is that these fish were able to grow in 0.1 p.p.b. endrin and, as will be pointed out later, showed some indication of reproductive activity.

The fish in 0.1 p.p.b. endrin appeared to be healthy, and behaved in a normal manner. They fed readily as soon as food was introduced. When they were examined after being killed, there were many fat deposits, especially around the abdominal cavity. These fish were much fatter than any of the other fish tested, including the controls. Whether there was any relation between this growth and the endrin is not known. This effect should be tested further with a larger number of fish.

If we assume similar growth rates for both sexes, the combined mean growth increments can be compared. In 0.5 and 0.4 p.p.b., the growth rates were lower than the control group by factors of approximately 0.71 and 0.68 respectively. These two concentrations were the ones in which a number of fish died before five specimens were found that would live in them, whereas the first five put into 0.1 p.p.b. lived without visible signs of poisoning. Reference to table 4 will show that in 0.5 p.p.b. 9 out of 14 or 64.3 percent died, and that 5 out of 10 or 50 percent died in 0.4 p.p.b. These percentages approximate the 96–hour TL_m values determined in the acute-toxicity testing. DeWitt et al. (1960) stated that in pheasants the concentrations of endrin producing acute and chronic effects were almost identical. A comparison of table 4 and table 9 indicates that the concentrations producing chronic and acute effects were very close together for the bluntnose minnow. In fact, those producing chronic effects are a little higher. This is thought to be attributable to the time of year, and to variations in individual fishes.

There were five deaths which occurred near the end of the chronic testing. A male in 0.1 p.p.b. appeared to be in breeding condition before losing equilibrium after 247 days in the test chamber. An autopsy showed excessive stored fat in all body parts, and sections of the liver revealed almost complete fatty vacuolation of the liver. When sections of the spleen, kidney, heart, and brain were compared with sections from normal tissue, no difference was noted.

Four females from the other test chambers died in the last 20 days of the test. They exhibited bulging eyes and rapid ventilation rates before death. The sectioned viscera appeared normal, the ovaries were well developed, and there were considerable fat deposits.

Sections of the liver and gonads of all fish which were living at the end of the test were prepared using the paraffin method and staining with hemotoxylin and eosin. Most of the fish, except those in 0.4 p.p.b., had considerable lipid deposits in the cells of the liver. In some cases, 90 percent of the surface area of a section was blank space, which I assume represents lipid material removed in the alcohol dehydration process. Sections of liver from fish recently taken from the stream did not show such fat deposits. In view of the low liver-fat content found in fish in 0.4 p.p.b. (which were in the test chambers for shorter periods of time) and in fish taken directly from the stream, it seems probable that this fatty vacuolation was caused by a high lipid content in the diet.

The effect of diet and reproductive condition on stored fat is discussed by W. S. Hoar in Brown's Physiology of the Fishes (1957). According to this author, changes in liver size due to a change in fat content, and correlated with the seasons, has been observed in several species. Apparently, a high fat content is normal for some fishes, but whether or not the condition found in these fish was normal is not known.

One male in 0.4 p.p.b., one in 0.1 p.p.b., and one in the control group had spermatids in the testes and moderately well developed tubercles on the head. The other males had both primary and secondary spermatocytes in the testes. Fighting was common among the males in 0.1 p.p.b., indicating sexual activity.

All the females had ovaries in a state of maturity comparable to fishes taken in the stream. Several females in TB–6, TB–7, and CB–10 showed distention of the abdomen resulting from ovarian development. It appears, therefore, that endrin in concentrations of 0.5 p.p.b. or less will not affect gonadal development in individuals which can tolerate such concentrations. More data are needed on this aspect of the subject before conclusions can be reached. It must be emphasized that only a fraction of the fish were able to live in

0.5 and 0.4 p.p.b. One fish in each of these concentrations was slightly emaciated, but no other physical effects were noted.

Little difference in activity was noted between fish in 0.1 p.p.b. and the control group, but fish in 0.5 and 0.4 p.p.b. rarely stayed under the rocks. On the other hand, the fish in 0.1 p.p.b. and in the control chamber normally stayed under the rocks during daylight hours. After sundown, all fish rested quietly on the bottom with little movement. Even fish having convulsions became quiescent after the room was darkened.

The increase in activity mentioned above could have a very detrimental effect on the fish in their natural environment. If sublethal concentrations of endrin were present in a natural body of water, they could quite possibly cause the fish to leave their normal habitat niche and be subjected to predation and the effects of current. The bluntnose spawns under objects, and such an effect on the activity could upset the spawning behavior and result in infertile eggs in this species. It is also important to bear in mind that even though the gonads were maturing, spawning did not occur, so that it cannot be concluded that endrin had no effect on spawning. These are matters that must be investigated before any conclusions can be drawn about the effects of sublethal concentrations on reproduction.

CHRONIC-TOXICITY TEST ON GUPPIES

Methods and equipment

This experiment was conducted simultaneously with the chronic-toxicity test on bluntnose minnows, from April 11, 1959, to October 24, 1959, under the same conditions. The water chemistry was the same as described earlier. The only differences were in the number and size of test chambers, and the endrin concentrations used. Guppy test chambers were 10 by 12 by 6 inches and held approximately 9 liters at test level; the flow rate was 6 ml. per minute. Paired chambers (designated by the letters "A" and "B" after the number) were fed by one reservoir and emptied through a common constant-level chamber. Test chambers were half as large as those used for bluntnose chambers; two test chambers could be supplied for the same period of time by one reservoir of the same size as those used for the bluntnose minnow tests. Six pairs of chambers (five pairs of test chambers and one pair for controls) were used in these tests.

The chronic-toxicity test was begun by assigning five 1- to 7-day-old guppies at random to the test chambers. Initially, endrin concentrations of 0.9, 0.75, 0.5, 0.25, and 0.1 p.p.b. were used. It was soon apparent that 0.9 and 0.75 p.p.b. were too high, so a 0.4 p.p.b. concentration was included. The guppies used were produced from breeders obtained from a local tropical-fish store.

Results

Before proceeding with the discussion of the data, it must be stated that the guppy test data should be interpreted cautiously because the animals became diseased, which probably affected some of the results. I first became aware of this disease early in September when the females in the control group began to appear dark in color, behave abnormally, and lose weight. They swam slowly about and stayed near the bottom, frequently with the head lower than the rest of the body. Immediately before they died, their abdomens became distended, their scales stood perpendicular to the body, and the fish came to the water surface. The same symptoms appeared in fish in other chambers and were followed by death. Examination of the dead fish revealed that the kidneys were enlarged and that there was an edema of the body cavity. Axelrod and Schultz (1955, p. 142) describe a disease called dropsy, which has symptoms similar to those observed in these test fish. The cause of this disease is thought to be a primary virus infection accompanied by a secondary bacterial infection. Upon checking back in my records, I found that some of the recorded descriptions of dying fish indicated that deaths may have been due to the disease as far back as the first of August. The disease seemed to affect sexually maturing females first and then the males. Because of this disease, the chronic study on the guppies had to be discontinued on October 24. 1959.

The question arises how the results of the guppy tests should be treated. Table 6 lists the fish which died in 0.9 and 0.75 p.p.b. Since the fish in these tests did not live for long, and since these tests were completed at least a full month before the disease was first observed, the results are probably reasonably accurate. At the time these tests were conducted, all fish in all chambers appeared healthy and normal, and those in the lower concentrations of endrin and in the control chambers were growing normally.

Table 7 presents the data for the lower concentrations of endrin tested. Here the disease no doubt was an important factor. For instance, in 0.25 p.p.b. there is a rather uniform progression of deaths with time; this is probably due to the disease, since the more usual pattern of deaths due to endrin, in both species, was numerous deaths within the first few weeks and then long periods before other deaths occurred. In view of the disease factor, no data have been given on growth rates, other than to indicate the fishes which attained sexual maturity.

Perhaps the most significant point to be inferred from the data is that if diseased fish were able to grow and reach sexual maturity in various concentrations from 0.5 to 0.1 p.p.b., then normal fish ought to survive and grow in at least those concentrations, if not in slightly higher concentrations.

Discussion of data

Inspection of the data given in table 6 for the two highest concentrations indicates that the adults were significantly more tolerant than the young. The reasons for this increased tolerance in the larger fish are not known.

A comparison of mean survival times in all concentrations shows an inverse relation between concentration and survival. If the mean survival times (tables 6 and 7) are plotted against the logarithm of the concentration, the points fall along a straight line, which is often the case in toxicological studies.

TABLE 6.—*Survival of guppies in 0.9 and 0.75 p.p.b. endrin*

Initial age	Survival time of each fish in days	Mean survival in days
0.9 p.p.b. concentration of endrin:		
1–7 days	3–3–3–3–2	2. 8
1–7 days	3–4–4–5–6	4. 4
1–7 days	4–4–4–4	4. 0
1–7 days	4–4–4–4–4	4. 0
Adult males	7–9	8. 0
Adult females	8–11–13	10. 6
Mean for 1–7 day fish		3. 8
0.75 p.p.b. concentration of endrin:		
1–7 days	3–3–4–4	3. 5
1–7 days	11–16–18–18–18	16. 2
1–7 days	3–3–3–4–4	3. 4
1–7 days	3–3–4–4	3. 5
Adult males	8–10–18–24	15. 0
Adult females	11–14–17–20	15. 5
Mean for 1–7 day fish		6. 6

The comparison of mean survival does not give a true picture of the toxicity of endrin, however. It was mentioned earlier that most deaths occurred at the beginning of the test period. This is es-

TABLE 7.—*Survival of guppies in 0.5, 0.4, 0.25 and 0.1 p.p.b. of endrin*

Initial age	Number of days each fish survived	Mean survival in days**
0.5 p.p.b. concentration of endrin:		
1-7 days	4-4-4-5-172*	4.3
Do	4-5-6-6-6	5.4
Do	6-6-7-8-8-9-9-9	7.8
Do	5-6-7-7-8-9-9-9-10	7.8
Do	5-5-6-6-7-7-7-11-14-28	9.6
Do	13-17-20-122*-122*-122*-122*-122*-122*-122*	16.6
Do	8-11-12-13-18-24-24-25-41-41	21.7
Do	25-45-122*-122*-122*-122*-122*-122*-122*-122*.	35.0
Do	5-8-11-82-101	41.4
Do	5-105-106♂-120♂-193♂	105.8
Adult males	25-26-24-154*	25.0
Adult females	31-39-40-44-154*-154*	38.5
Mean for 1-7 day fish		25.5
0.4 p.p.b. concentration of endrin:		
1-7 days	6-10-15-30-32-32-137*-137*-137*.	20.8
Do	7-10-26-42-92-129♀-137*♀	51.0
Do	62-83-87-101*♀-101*♀-101*♀-101*♂-101*♂-101*♂-101*♂.	77.3
Mean		49.7
0.25 p.p.b. concentration of endrin:		
1-7 days	5-80-146♂-163*♂-175*♂	77.0
Do	55-61-163♀-196*♂196*♂	93.0
Do	12-44-45-60-64-70-81-83-97-122*.	61.8
Mean		77.3
0.1 p.p.b. concentration of endrin:		
1-7 days	108-102*-122*-122*-122*-122*-122*-122*-122*.	108
Do	41-70-132♀-188♀-196*♂	108
Do	110♀-118♀-169♀-196*♂	132.3
Mean		116.1
0 p.p.b. concentration of endrin:		
1-7 days	12-70♀-159♀-191♂-196*♂	108
Do	168♀-170♀-177♂-134♂	162.8
Mean		135.4

*Fish were killed at the end of the test.

**Fish which were living at the end of the test are not included in the calculation of mean survival.

pecially noticeable in 0.5 p.p.b. of endrin. An inspection of the individual survival times in table 7 reveals that, in general, fish which survived 30 days were likely to survive much longer. This pattern would be even more definite if the disease had not been present. A discussion of the cumulative effects of endrin will be found in the section beginning on page 29.

Such evidence, plus data on sublethal effects presented in a later chapter, indicate that the threshold falls in a rather narrow range of concentrations near 0.5 p.p.b. endrin. Above this threshold the fish die, and below it the fish live with few physical effects.

The data are too scanty to draw any conclusions concerning a sexual difference in tolerance. On the basis of a cursory examination of the tables, it seems that there is no appreciable difference between the sexes.

The only data on reproduction were obtained from the adults used in the tests. Some of the adult females were pregnant when placed in the test solutions, but of six females which bore young, only one bore a second litter. This female was in 0.5 p.p.b. of endrin. There were six in the first litter, all normal in appearance. In the second litter, 6 of the 12 had large yolk sacs and were probably premature. However, the rate of survival of these young was about the same as for normal ones. The young born from the other five females (two in 0.75 p.p.b. and three in 0.5 p.p.b.) had survival rates similar to normal young also. (No adult females were put into the two lower concentrations.) Whether the effect of the endrin is on the egg or on the sperm is not known. Males frequently attempted to copulate with the females. Of the females which grew to sexual maturity in concentrations of endrin, most contained well-developed eggs when they died. (Death presumably was due to the disease.)

Fish were not measured during the tests, so their growth rates are not known. However, the two highest concentrations definitely retarded or stopped growth. Several fish in 0.5 p.p.b. grew very little. One was 126 days old when killed and measured only 8 mm. standard length, this size being no larger than that of many normal guppies at birth. Since I have noted similar stunting in the past under normal aquarium conditions, this may not have been caused by endrin.

The symptoms of poisoning were very

similar to those described for bluntnose minnows, with one notable exception. Guppies went to the bottom, whereas bluntnose minnows went to the surface. This was unusual, because bluntnose minnows are bottom feeders and guppies are surface feeders.

No data were obtained on increased resistance in offspring of fish chronically exposed to endrin. I planned to run tests on offspring of fish raised in endrin to determine whether there was increased tolerance, but unfortunately no young were obtained for these tests. The only available data on this aspect of the subject come from observations of fishes put into test solutions. Without exception, the fish that survived for long periods in 0.5 p.p.b. endrin went through a period of poisoning with symptoms like those described on page 12, for the females in TB–7. Like the female bluntnose, the guppies returned to normal appearance and behavior after a period of time. Hyperactivity and slight hypersensitivity were the only abnormal types of behavior noticed. If after the initial period endrin's effect is all or none, as I have postulated earlier, then one would not expect tolerance to increase to any great extent.

ACUTE-TOXICITY TESTS ON BLUNTNOSE MINNOWS

Objective

The objective of this part of the investigation was to determine whether there were significant differences between the various size groups in their acute tolerances to endrin.

Methods and equipment

Continuous-flow tests were used to determine the endrin-toxicity values for various size groups of bluntnose minnows. The necessity of using a continuous-flow system as opposed to a static or nonflowing system is often questioned. Henderson and Tarzwell (1957) suggest a static test, probably because it is quicker and easier. When testing volatile substances there is a loss due to evaporation. If aeration is necessary (as it often is if dissolved oxygen is to be maintained at 5 p.p.m. or higher) the loss may become appreciable. When aeration is needed, the above-mentioned authors suggest a slow stream of pure oxygen, but the use of oxygen is very bothersome. Other problems arising in static testing are the accumulation of excretory products, especially carbon dioxide, and the growth of bacteria. Continuous-flow systems hold these factors to a constant minimum, and while these factors are not eliminated in absolute terms, they are eliminated as variables. If aeration is necessary, the continuous-flow system has a distinct advantage.

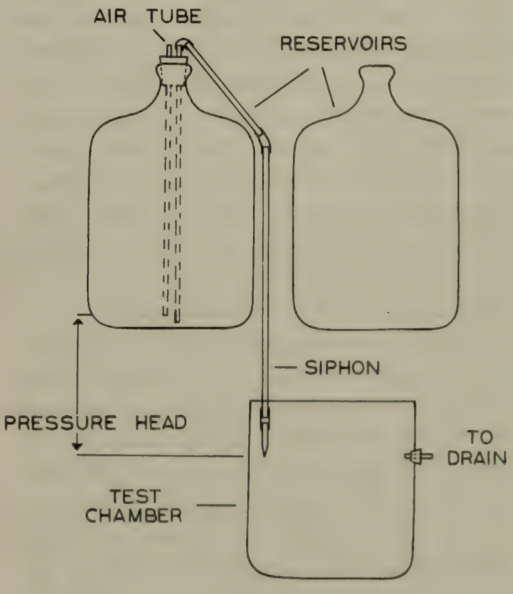

Figure 4.—Diagram of continuous-flow system used for acute testing.

Sometimes the amount of a toxic substance absorbed by the test animals in static tests becomes significant, and the continuous flow will offset this effect also. In this test I suspected that the differences between size groups would be slight, if they occured at all. The continuous-flow system was used, therefore, to minimize the lowering of the endrin concentration by absorption.

The test chambers were 5-gallon glass jugs cut off at a height of 12 inches. An overflow hole was drilled 6 inches from the bottom; the capacity at overflow level was approximately 9 liters. These test chambers were placed in a large water bath which was maintained at 22.2° C., plus or minus 1° C. These chambers were supplied from a 5-gallon glass jug reservoir placed on a rack above them. Water was siphoned from the reservoir, and the flow was controlled by means of a small glass jet. Constant pressure was maintained in the siphon by stoppering the reservoir and running the air tube to the bottom of the jug. In this way the pressure on the siphon was a head of water equal to the vertical distance from the end of the air tube to the end of the siphon. Pressure was independent of the water level so long as the level was above the end of the air tube. This system gave a very constant pressure as the jug emptied. All water lines were made of glass, with flexible joints made of tygon tubing. When the flow was 10 ml. per minute, one 5-gallon jug held a 32-hour supply.

There were two jugs for each test chamber, the siphon and air tube being transferred from one to the other. This made it possible to fill one jug 24 hours in advance of its use, which was sufficient time for the water to warm to room temperature and for the free chlorine to escape. Each test chamber was aerated at a uniform rate with a glass air jet. This aeration also ensured adequate mixing. Five test chambers were used, one as a control and four as test chambers.

A stock solution of endrin in alcohol was made for each test chamber, using the initial stock solution of 0.1 mg. of endrin per milliliter of 95-percent alcohol. Additional alcohol was added so that equal volumes of the stock solutions added to each reservoir would give the desired concentration. This volume was 0.00001 ml. of stock solution per milliliter of water. This gave a uniform concentration of alcohol in water in each chamber. The measured amount of solution was placed on a glass stirring rod and lowered into the reservoir. The same volume of pure 95-percent ethyl alcohol was added to the control. Five fish were assigned to each testing chamber by the use of a table of random numbers.

The tests followed the basic routine described by Henderson and Tarzwell (1957). Fish were held for at least 2 weeks at 22.2° C. before testing. They were not fed for 48 hours prior to testing nor during the test. Alkalinity, pH, dissolved oxygen, and free carbon dioxide were measured at the beginning of the test, again when the fish began to die, and finally at the end of the test. The 96-hour TL_m values were determined by straight-line graphic interpolation. The concentration of endrin was plotted on a log scale and percent survival on a linear scale. A line was drawn between the two points bracketing the 50-percent survival concentration, and the point on the concentration scale at which this line crossed the 50-percent survival level was read as the 96-hour TL_m value. Percent survival was calculated as the percent living in each chamber after exactly 96 hours. In all cases fish were clearly alive or dead, and there was no problem with borderline cases.

The chemical properties of the water are listed in tables 1 and 8. All determi-

TABLE 8.—*Chemical characteristics of the water in the acute-toxicity test chambers*

[All values, except pH, are expressed in p.p.m. These values are based on 27 determinations]

Characteristic	Range	Mean
Dissolved oxygen	6.4–9.1	7.5
Free carbon dioxide	1.0–0	.5
Phenolphthalein alkalinity	0–14.0	4.1
Methyl orange alkalinity	11.0–32.0	23.2
Total alkalinity	17.0–33.0	27.3
pH	*7.5–9.0	8.4

*The high pH was present only for the first few minutes of the test until aeration reduced it.

nations were made by use of techniques listed in Welch (1948). The unmodified Winkler method was used for dissolved oxygen, and the pH determinations were made colorimetrically as described earlier.

All fish used were collected from the same pool in Rocky Fork Creek as were the animals used in the chronic-toxicity study. With few exceptions, all fish were heavily parasitized by tapeworms (Plerocercoids of *Proteocephalus*) in the mesenteries. These parasites did not noticeably affect the fish, all of which appeared very healthy. On four occasions large numbers of fish (200 to 500) were brought into the laboratory, and each time not more than 10 died during the acclimation period. These died within the first few days, probably as a result of injury when captured. No deaths occurred in the control chamber during the testing. The fish were fed ground dog-chow checkers during the acclimation period before testing. Only three size groups were available, with mean sizes of 29.8, 40.6, and 59.8 mm. standard length; these are designated size groups 1, 2, and 3 in table 9. Fish were preserved in 10-percent formalin and were measured to the nearest millimeter, standard length.

Results and discussion

Table 9 summarizes the data and analysis of variance for the acute tests (Snedecor, 1946). Appendix 1 lists the raw data from the tests. Each 96-hour $TL_{\overline{m}}$ value is based on five fish in each of four concentrations. Eight preliminary tests were run before a suitable range of concentrations was found.

TABLE 9.—*Data and analysis of variance for acute-toxicity tests on bluntnose minnows*

A. 96 hour $TL_{\overline{m}}$ values

[Values expressed in p.p.b. endrin in water]

	Size group 1 (29.8 mm. mean S.L.)	Size group 2 (40.6 mm. mean S.L.)	Size group 3 (59.8 mm. mean S.L.)	Sum	Mean
Test 1	.26	.35	.51	1.12	.37
Test 2	.28	.24	.43	.95	.32
Test 3	.28	.28	.46	1.02	.34
Sum	.82	.87	1.40	3.09	--------
Mean	.27	.29	.47	--------	.34

B. Summary table for analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square
Total	8	0.0786	------------
Group	2	.0689	0.0345
Test	2	.0049	.0025
Discrepancy	4	.0048	.0012

F value for groups=28.75.
F_{01} value with 2 and 4 degrees freedom=18.00.

The *F* value for between groups exceeds the value of *F* at the 0.01-percent level, with two and four degrees of freedom, indicating a probable difference in the tolerance of various sizes. This difference, while detectable, is not large enough to be of importance in setting safe levels for natural bodies of water.

No statistical test is available to determine the effect of acclimation period on the toxicity in this case. An inspection of table 9 indicates that the variation within any group is not nearly as great as between certain groups. As an example, the first $TL_{\overline{m}}$ value listed under size group 3 is for fish held 14 days before testing. The third value in the same group is for fish held 57 days before testing. Both groups were from the same collection of January 1, 1960. Reference to appendix table 1, tests S–11 and S–17, will show that the mean sizes of these two groups were 63.4

and 58.9 mm., standard length. The difference in $\mathrm{TL}_{\overline{m}}$ values could be due in part to size differences. Since the maximum variation of $\mathrm{TL}_{\overline{m}}$ values within any group is not more than 0.09 p.p.b., the length of the acclimation period beyond 14 days (recommended by Henderson and Tarzwell, 1957) does not introduce variation of practical significance.

The effect of temperature on toxicity was investigated by Iyatomi et al. (1958), who found that a decrease of 20° C., from 27° C. to 7° C., caused the tolerance of 7- to 8-centimeter carp to increase by a factor of 28. It is doubtful whether this difference would be as great in longer exposures. The effect of temperature should be investigated further.

From table 4 and appendix table 1 it is clear that the fish used in the chronic-toxicity test were more tolerant to endrin than those used in the acute-toxicity tests. At first it seemed most probable that the lower tolerances of the fish in the acute tests were caused by physiological changes in the fish as a result of the colder weather, since all fish used in the acute tests were collected on December 6, 1959, and January 1, 1960. If this were the case, one would expect the acute $\mathrm{TL}_{\overline{m}}$ values to change as the fish were held for longer periods. This does not appear to be the case, as mentioned above.

There are three other possible causes for this difference: (1) The fish used in the chronic-toxicity tests were not parasitized and may have been in better physical condition. (2) The fish used in the chronic-toxicity tests were from smaller populations in less crowded conditions. The stream population was extraordinarily large when the fish used in the acute-toxicity tests were collected. Langlois (1951) reported that bluegills living in crowded conditions were less hardy. (3) The toxic effect of endrin may be affected by the reproductive physiology. Whatever the cause of the reduced tolerance, the effect should have been the same for all groups, and therefore the difference found between size groups should be a true one.

Since bluntnose minnows cannot be sexed by external characters, except during the breeding season, any sexual differences in the tolerance to endrin must be obtained from the acute toxicity tests. Sexually mature fish were used in five acute-toxicity tests, and the mean survival time of both the 56 females and the 44 males used in these tests was 3.2 days. Treon, Cleveland, and Cappel (1955) and others found that in mammals there is a sexual difference in tolerance to endrin.

EFFECT OF ENDRIN ON OXYGEN CONSUMPTION

Objective

The objective of this part of the investigation was to determine whether endrin has any effect on the rate of oxygen consumption. The large increase in the ventilation rate indicated that the fish might be using more oxygen.

Methods and equipment

A system similar to the one described by Keys (1930) was modified and used for these oxygen-consumption tests. The basic plan was to measure the difference in the dissolved oxygen in the effluent of two closed chambers, one containing a fish and one without a fish. A reservoir and constant-head chamber were used as a water supply for the oxygen tests (fig. 5). The water was aerated with compressed air in the constant-head chamber. Two 1-quart glass jars with stoppers served as test chambers. They were submerged in a water bath which was main-

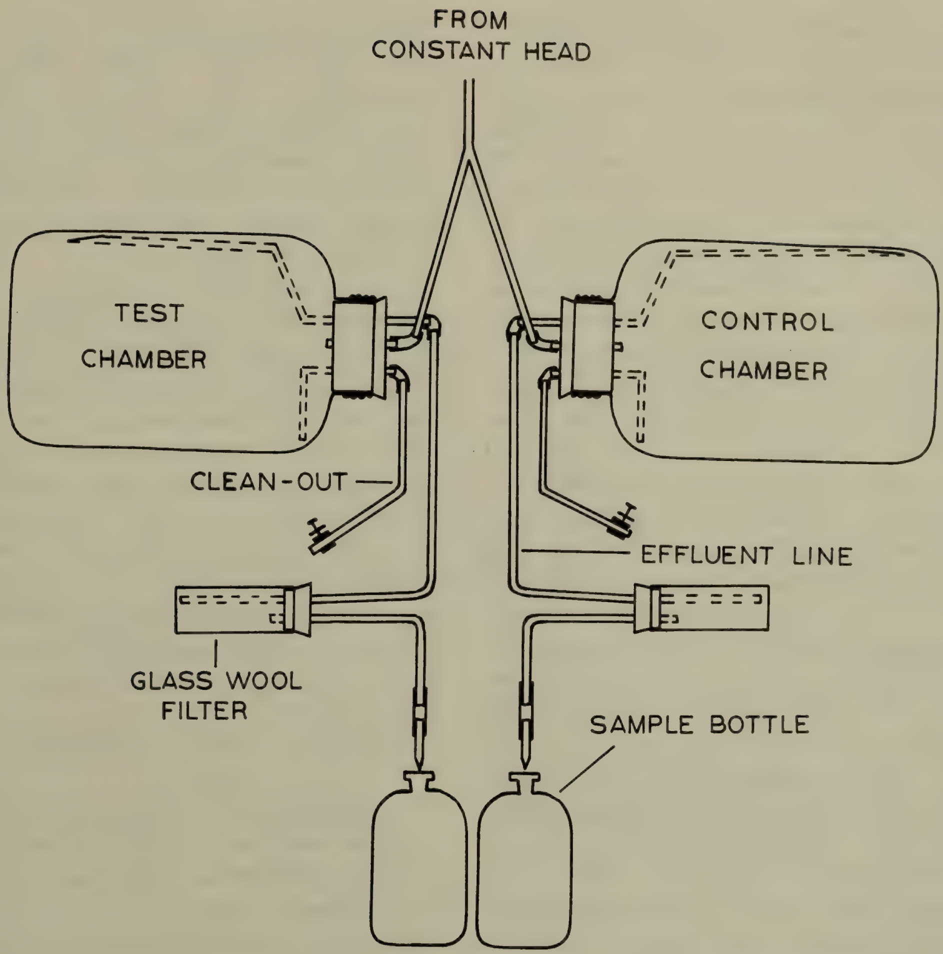

FIGURE 5.—Diagram of apparatus used to measure oxygen consumption.

tained at 24° C. by a commercial aquarium heater. Temperature stayed within the range of 23° to 24° C. Each jar was placed on its side, and water was introduced into the stoppered end through a siphon and removed by another syphon leading from the top of the closed end. The test-chamber effluent was filtered through a glass-wool filter which was cleaned every 2 days to prevent bacterial growths. Flow rates were regulated by means of small glass jets, and were measured with a 10-ml.-capacity graduated cylinder. A flow rate of 6 ml. or 12 ml. a minute was used, depending on the size of the fish being tested. If a flow rate of 12 ml. a minute was used, the flow was measured for 30 seconds three consecutive times and the average of the three readings was used. If a flow of 6 ml. a minute was used, the flow was measured one time for a period of 1 minute. Measurements of flow rate were made immediately after the sample for oxygen determination was taken. The dissolved-oxygen concentration of the effluent from the test chamber was approximately 4 p.p.m. On three occasions it went down to 3 p.p.m.

Sample bottles, 125-ml. capacity, were placed under the glass jets at such a level that when the bottle was overflowing only $\frac{1}{16}$ inch to $\frac{1}{32}$ inch of the glass jet was beneath the surface of the water in the bottle. In this way the sample bottles could be filled and left under the jets all the time without reducing the flow or admitting oxygen to the water. Sample bottles were left under the water jets for a minimum of 4 hours before the dissolved oxygen was determined. This method was also advantageous in that the sample could be removed before my presence disturbed the fish and caused its oxygen consumption to increase. The 125-ml. sample was representative of the preceeding 10 minutes, or 20 minutes, depending on the flow rate, and therefore small increases of oxygen consumption for short periods did not disrupt the determinations. Methylene blue was used several times to determine whether the water in the sample bottles was being completely mixed by the water stream. It was found that the stream of water from the jet went to the bottom of the sample bottle and then moved outward and upward. By the same method it was found that good mixing was also taking place in the test chambers.

The Winkler method, with a few minor changes, was used for dissolved-oxygen determinations. All volumes of the solutions used were reduced by half, and N/100 sodium thiosulfate was used instead of N/40, in order to obtain greater accuracy. One hundred milliliters of the sample were measured with a volumetric pipette and titrated. Since oxygen consumption was based on the difference between the two sample concentrations, no correction for loss of oxygen due to addition of reagents was necessary.

In the first tests, the fish were fed ground dog-chow checkers daily through a T-joint in the water line. In later tests, feeding was discontinued because it was felt that it introduced too much variation into the consumption rate. This was true even in readings taken 24 hours after the fish were fed. See figures 6 through 12.

The fish were weighed in water on a beam balance to the nearest tenth of a gram before the test and were measured and sexed after the test. All fish used for oxygen-consumption tests were collected from the same location as those used in the other testing. They were held at 18° to 20° C. before being tested.

After a fish had been weighed, it was placed in one test chamber and left for a few hours, usually overnight. Then serial measurements of its oxygen consumption were begun. Measurements were made every 24 hours in tests O–1, O–2, O–4, and O–5, and at about 12 hour intervals in tests O–6, O–7, and O–8. Disturbances were at a minimum in the basement where the tests were conducted. There were few vibrations, because the floor was concrete. The actual formula used for determining oxygen consumption was as follows:

$$\frac{C \text{x} 0.8 \text{x} F \text{x} 0.698}{W} = \text{ml. of oxygen per gram per hour.}$$

C is the difference in titration values between test and control samples.

0.8 is a factor for converting titration values to mg.

F is the flow rate in liters per hour.

0.698 is the factor to convert milligrams of oxygen to milliliters of oxygen under standard conditions.

W is the weight of the fish in grams.

Results

Table 10 lists the mean consumption rates for each fish tested. In the first four tests, the fish were held in the test chamber for 5 to 15 days in an attempt to get a constant consumption reading. There was little change in consumption after the first few hours, however, so in later tests the period of time before endrin was introduced was shortened.

Although with one exception the mean oxygen consumption is higher in the presence of endrin than under normal condi-

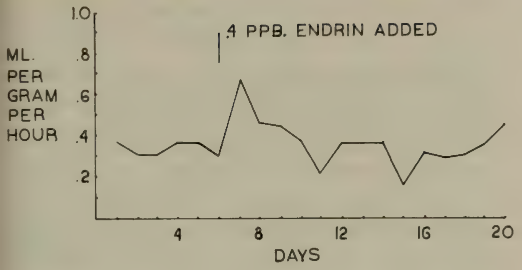

FIGURE 6.—Test O–1. Oxygen consumption of a 0.7-gram female bluntnose minnow, 38 mm. standard length. Fish was fed in the evening and consumption was determined at 7 a.m.

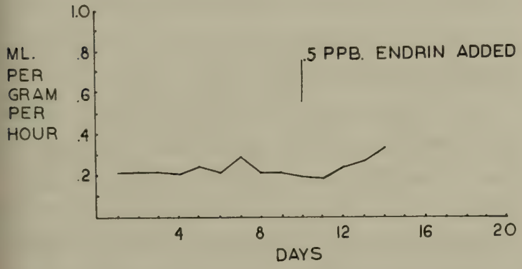

FIGURE 7.—Test O–2. Oxygen consumption of a 4.2-gram male bluntnose minnow, 70 mm. standard length. Fish was fed in the evening and consumption was determined at 7 a.m.

tions, the difference is not over 0.06 ml. per gram per hour in any case. Usually the difference between means is less than the daily variation.

Figures 6 through 12 represent graphically the oxygen consumption for all fish tested. In test O–8, figure 12, oxygen consumption was measured every 4 hours for 16 hours after endrin was added, but no variation was found.

Discussion

Harvey and Brown (1951) report that compounds related to endrin (aldrin and dieldrin) cause a fivefold increase in oxygen consumption when injected into the cockroach. They found a latent period of 200 to 300 minutes before oxygen consumption reached the peak and then tapered off until death. No such increase was noted in the bluntnose minnows; such increases as occurred were 25 percent or less above normal. Fry, in The Physiology of Fishes (Brown, 1957), gives data on goldfish which show a fourfold increase in oxygen consumption due to activity. The slight increase found here in fish exposed to endrin would be most logically attrib-

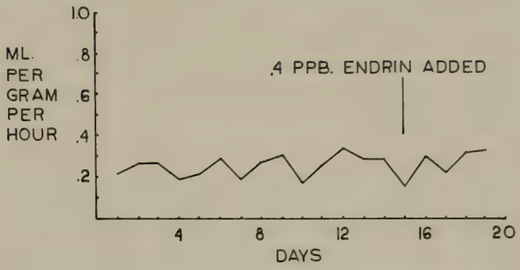

FIGURE 8.—Test O–4. Oxygen consumption of a 5-gram male bluntnose minnow, 73 mm. standard length. Fish was fed approximately 24 hours before consumption was determined at 7 a.m.

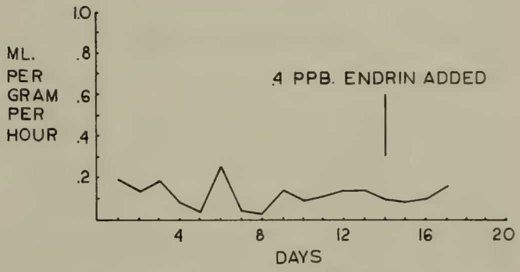

FIGURE 9.—Test O–5. Oxygen consumption of a 2-gram female bluntnose minnow, 56 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m.

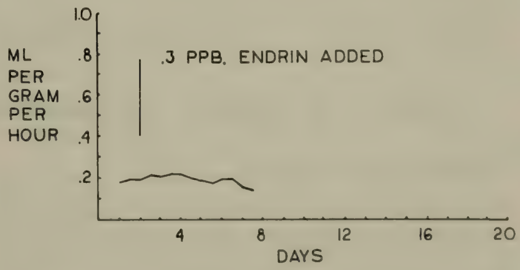

FIGURE 10.—Test O–6. Oxygen consumption of a 2.6-gram female bluntnose minnow, 60 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m. and 6 p.m.

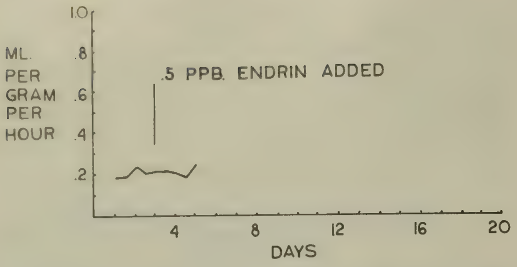

Figure 11.—Test O–7. Oxygen consumption of a 3-gram male bluntnose minnow, 61 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m. and 6 p.m.

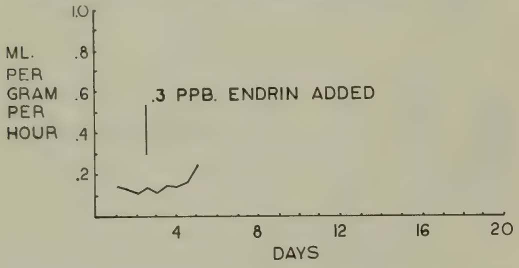

Figure 12.—Test O–8. Oxygen consumption of a 3-gram male bluntnose minnow, 61 mm. standard length. Fish was not fed. Consumption was determined at 7 a.m. and 6 p.m.

Table 10.—*Mean oxygen-consumption rates of bluntnose minnows*

Tests O–1 and O–2: Fed 12 hours before readings were taken.
Test O–4: Fed 24 hours before readings were taken.
Test O–5: No feeding for 12 days before endrin was added.
Test O–6: No feeding, killed after 6 days in endrin.
Test O–7: No feeding for 1 day before endrin was added.
Test O–8: No feeding for 9 days before endrin was added.

Test No.	Weight in grams	Standard length in mm.	Sex	Normal consumption ml/g/hr.	Consumption in endrin ml/g/hr.
O–1	0.7	38	F	0.35	0.37
O–2	4.2	70	M	.22	.25
O–4	5.0	73	M	.23	.28
O–5	2.0	56	F	.11	.11
O–6	2.6	60	M	.18	.19
O–7	3.0	61	M	.20	.21
O–8	3.0	61	M	.13	.16

uted to the increased movement due to tremors and convulsions.

The insignificant increase in oxygen consumption is surprising in view of the great increase in ventilation rate. No counts of ventilation rates were feasible on small fish with such high rates, but it appeared that the fish were ventilating at a maximum rate. Since the ventilation rate increased greatly, while the rate of oxygen consumption did not increase significantly, and since the ability of the fish to work was not affected, it seems that the apparent respiratory distress must be due to a stimulus other than a deficiency of oxygen in the tissues. The effect could be attributed to accumulation of metabolites.

EFFECT OF ENDRIN ON SWIMMING OF BLUNTNOSE MINNOWS

Objective and method

The object of this experiment was to determine whether endrin reduces the ability of fish to carry on muscular exercise. The fish were forced to swim against a current in a current tray (Mount, 1959). The tray was 2 feet wide by 4 feet long by 6 inches deep, with a divider in the center. A paddle wheel powered by a pump motor propelled the water around the tray. The tray was modified slightly in that the corners were rounded, thereby increasing the current to approximately 9 inches per second. Eddies and slow areas were eliminated by placing bricks on the bottom under them, so that the fish could not rest in currentless water.

Test fish were specimens from the highest concentrations of the acute-toxicity tests in which four or five of the fish had survived. Control fish were taken from the control chamber. Both groups of fish had not been fed for 6 days before testing. All testing on both sexes was at 22.2° C. The test fish were distinguished from the control fish by clipping opposite pelvic fins on each group. The paddle wheel was started and a periodic check was made to remove any dead or exhausted fish.

Results

The first two tests were run on fish 30 to 43 mm. standard length, which had been in a concentration of 0.2 p.p.b. endrin for 4 days. In both tests the test fish were tremulous. In the first test one fish died after 3.5 hours. The other four test fish, the five controls, and all five test fish and the five controls in the second test swam against the current for 48 hours, at the end of which the test was discontinued.

In the third test, four fish from the test chamber containing 0.4 p.p.b. endrin were used. They were at the surface and in the latter part of the convulsion stage when the test was begun. They ranged in size from 49 to 75 mm. standard length. Five hours after the current was started one died, and after 8 hours another died. The other two test fish and the five controls were alive and normal after 48 hours.

The last test was run on five fish from the test chamber containing 0.4 p.p.b. endrin. The fish were in the early stages of convulsions. One died after 24 hours, but the remaining four test fish and the controls lived for 48 hours. These fish ranged in size from 56 to 59 mm. standard length.

Discussion

It is my opinion that the three fish which died in the last two test runs would have died if they had been in quiet water. One fish which died in the first test may have died from exhaustion or may have been injured while being handled. In tests 3 and 4, the fish stopped having convulsions as soon as the current was started. In test 3 I turned the current off momentarily after about 30 minutes, and they began having convulsions again.

The only conclusion which can be drawn is that in acute poisoning endrin has little effect on the ability of fish to swim against a current. However, it is possible that if the fish could have been tested in a much swifter current in which even normal animals became exhausted, the test fish would have become exhausted first.

PORT OF ENTRY INTO AND DISTRIBUTION OF ENDRIN IN THE FISH'S BODY

Objective

The extreme toxicity of endrin to fish causes one to wonder what the specific action of endrin might be. The symptoms appearing during the period of poisoning indicate that the effect is mediated through the nervous system. In an effort to gain insight into this problem, an experiment was conducted to determine the point of entry and the movement of endrin through the body.

Methods

Davidow and Sabatino (1954) describe a method of steam distillation by which very low concentrations of endrin were recoverable. When a solution or material containing an endrin residue is boiled in an aqueous solution or slurry, and the steam is condensed and collected, most of the endrin is found in the first 200 ml. of distillate.

With slight modification, this method was employed successfully. A 1,500-ml. Erlenmeyer flask was connected to a Liebig condenser. The material or solution to be extracted was placed in the flask. A fraction of the volume was collected as distillate and the bioassay was run in the distillate. After experimenting with solutions of known concentrations, it was found that collection of 250 ml. of distillate, regardless of the original volume, gave satisfactory recovery. By collecting 250 ml. of distillate from 1,000 ml. of solution known to contain 0.5 p.p.b. endrin, it was possible to get a concentration of 1.5 p.p.b. or more in the distillate, as determined by a fish bioassay. This represents a recovery of 75 percent or more.

Several bluntnose minnows (with a total weight of 8.1 grams) which had died in 0.5 p.p.b. endrin in 5 days were cut into small pieces and put into a flask with 500 ml. of water. Two hundred and fifty milliliters of distillate were collected, and a small bluntnose minnow was put into this solution. At the same time, another small bluntnose minnow was placed in 250 ml. of a solution containing a known concentration of 2.0 p.p.b. endrin. The fish in the distillate died in 11 hours and the one in the known concentration in 15 hours, which indicated a concentration of over 2.0 p.p.b. in the distillate. Another fish was added to the distillate, and it died in 10 hours. Other distillations were made with similar results. Fish not exposed to endrin were also distilled, but no deaths occurred when the distillates were assayed. From this evidence it was concluded that the technique was reliable. It was also more sensitive than the best chemical test, which is only accurate to 5 p.p.b. Bioassay also has the advantage of being a test of toxicity, which would therefore be positive even if the endrin were slightly altered chemically within the fish or by the distillation. A chemical test might give negative results if the endrin were chemically altered (Klein et al., 1956).

No attempt was made to determine whether the endrin had been chemically altered. One purpose of this testing was to estimate how much endrin accumulates in the body, as an index to the potential danger of such fish in the food chains and to man. So long as the compound is toxic, it makes little difference what its specific form may be. Some insecticides are changed into different compounds. For example, aldrin is changed to dieldrin and stored in the fat (Bann et al., 1956). Kunze and Laug (1953) found that endrin "of a different chemical nature" occurred in the kidneys.

For the study of endrin's entry and distribution within the fish, large carp of approximately 40 cm. standard length were used. They were "snagged" from the Scioto River at Columbus with treble hooks. Only those not seriously injured were used for testing. They were maintained for 2 or 3 days before being put into endrin. In the test, a 30-gallon porcelain tub was used for each fish. Twice daily the tub was partially drained and 10 gallons of fresh solution were added. The temperature was approximately 20° C.; no attempt was made to control it. The test numbers in table 11 represent the following exposure times to endrin:

	Concentration in p.p.b.	*Exposure time in days*
Test 1	0. 0	0. 0
Test 2	10. 0	2. 5
Test 3	5. 0	4. 0
Test 4	5. 0	5. 0
Test 5	2. 5	28. 0

Test 1 served as a control to establish that no toxic substances other than endrin were carried over into the distillate. This fish was killed and distilled immediately after being captured. The fish in tests 2, 3, and 4 died; the fish in tests 1 and 5 were killed.

Immediately after death the fish were opened. The various organs were removed, weighed in water, mascerated, and placed one at a time in the flask. Five hundred milliliters of tap water were added to the flask and distilled until 250 ml. of distillate had been collected. The distillate was clear but had a smell of fish oil which disappeared after 5 to 10 hours at room temperature. All distillates were left at room temperature for approximately 12 hours before being assayed.

Each distillate was diluted with tap water so that a 250-ml. aliquot represented the extract from 10 grams of tissue. Thus if there were 100 grams of tissue, 2,250 ml. of tap water would be added to the 250 ml. of distillate, the solution mixed, and 250 ml. aliquot used for the bioassay. In this way errors in weighing tissue were minimized, and since each 250 ml. sample represented the extract from 10 grams of tissue, the times of exposure to the extract until convulsions set in (and therefore the concentrations) could be compared directly. Ten grams was selected as the basic weight because this was the usual minimum weight of the smaller organs.

The assay was carried out in 1-quart glass jars at room temperature with very gentle aeration, for a 48-hour period. Two hundred and fifty milliliters of various known concentrations were placed in similar containers for comparison. In the fourth test, enough endrin was added to each distillate and to the known concentrations to make a 0.5 p.p.b. solution. In the fifth test, enough was added to make a 1.0 p.p.b. solution. Endrin was added for two reasons: (1) It speeded the test but did not affect the results, since concentrations were determined by comparison. (2) It minimized the effect of individual variability which is less significant at higher concentrations. All fish used in the tests were bluntnose minnows, 20 to 30 mm. standard length, from the lot collected on January 1, 1960, for use in the acute-toxicity tests. As shown by the acute-toxicity testing (appendix table 1), fish in this size-range showed little variability in tolerance. Daphnia and insect larvae were not sensitive enough for assaying the concentrations. One fish was placed in each container for the test.

The onset of convulsions proved to be the most satisfactory end point. The time from the beginning of exposure until the onset of convulsions was quite uniform and predictable within a known concentration (see table 11), whereas that from the beginning of exposure until death was found to be much more variable. The latter took longer for some fish in 10 p.p.b. than for others in 5 p.p.b. Davidow and Schwartzman (1955) found the same variation in their bioassays with goldfish, and they too used convulsions as the end point. Tarzwell and Henderson (1956) used loss of equilibrium as an end point. Klein et al. (1956) used both convulsions and loss of equilibrium as end points. In this investigation, loss of equilibrium was more variable than onset of convulsions.

Results

Table 11 summarizes the results of the bioassays on the corp organs. In tests 2, 3, and 4, heart-spleen-blood (combined), digestive tract, kidney, and liver ranked highest in endrin concentrations. In addition, in test 5 the brain contained a measurable amount of endrin, as did the fat. In test 5, blood was tested separately and contained measurable amounts of endrin, but heart-spleen ranked higher.

TABLE 11.—*Bioassay of extracts of carp tissues using bluntnose minnows*

[Tests were conducted for a 48-hour period in the extract from 10 grams of tissue, except as noted. "Neg." indicates that no convulsion occurred in 48 hours]

	Hours until the onset of convulsions of bluntnose minnows				
	Test 1	Test 2	Test 3	Test 4[1]	Test 5[2]
Known concentration of endrin:					
15 p.p.b	--------	2.75	--------	--------	--------
10 p.p.b	--------	3.0	4.5	3.0	3.25
5 p.p.b	--------	4.0	6.5	4.0	6.25
2.5 p.p.b	--------	--------	7.5	7.0	10.75
Carp tissue extracts:					
Blood	--------	--------	--------	--------	8.25
Brain[3]	neg.	neg.	neg.	[4] neg.	[5] 6.25
Fat	neg.	10.3	neg.	neg.	8.75
Gills	neg.	neg.	neg.	neg.	12.25
Heart, spleen, blood	neg.	5.25	25.5	[6] 19.0	[7] 3.25
Digestive tract	neg.	7.25	13.0	40.0	10.75
Kidney	neg.	6.25	48.0	19.0	[8] 4.00
Liver	neg.	7.0	48.0	19.0	7.75
Muscle	neg.	9.0	neg.	neg.	neg.
Ovary	neg.	--------	neg.	neg.	neg.
Testes	--------	10.25	--------	--------	--------

[1] 0.5 p.p.b. endrin added.
[2] 1 p.p.b. endrin added.
[3] Includes some fat in tests 1, 2, 3, and 4.
[4] 8.5 g. tissue.
[5] 3.2 g. tissue.
[6] 9.5 g. tissue.
[7] 6.2 g. tissue.
[8] 6.5 g. tissue.

In previous tests, blood was included with these two tissues so that the total weight was approximately 15 grams; therefore, the distillate had to be diluted by one-half. This would tend to mask out a high concentration in the spleen or heart.

Some error is introduced by the variability of the assay animals, and by the small size of the sample. The four tests do present a consistent picture, however, and for this reason are considered reliable.

Discussion

On the basis of the data in table 11, it may be inferred that endrin is being carried away from the place of entry into the body by the blood. Of the organs assayed, excepting the gills, those having a rich blood supply contained the most endrin. The high concentrations found in the kidney suggest that endrin may be excreted through that organ. Other investigators (previously cited) have found significant damage in the kidney, further suggesting the importance of this organ in the excretion of endrin.

If the kidney is the organ excreting endrin from the body, the high concentration found in the digestive tract presents a puzzling question. The gills gave mostly negative results. (The one positive value may have been due to the endrin added to the distillate.) It seems logical that endrin would have been detected in the gills if it were entering there. It is more probable, in view of the evidence, that the digestive tract was absorbing the endrin. This hypothesis accounts for the negative results of the gill tissue and the positive results of the intestinal tissue.

Since the carp were not fed during the exposure, endrin could not have been taken up from the water swallowed in connection with food ingestion. The statement appears in Brown's Physiology of the Fishes (1957) that "Most of the water is taken in through the semipermeable gill and oral tissues, although some also crosses the less permeable body surface . . . The only other water intake in fresh-water fishes is that accompanying or bound in the food swallowed." There is reason to doubt this statement, at least in the case of some species of fresh-water fish. Both Ellis (1937) and Allee and Frank (1948) have reliable data to the contrary, that fresh-water fish may drink sizable amounts of water. The latter workers showed this to be the case in goldfish. Since the carp is closely related to the goldfish, it is not unreasonable to assume that carp may also drink water, even when not feeding. Allee and Frank give a complete summary of the literature on water intake by fresh-water fish.

Further evidence that the digestive tract may be the port of entry is furnished by Iyatomi et al. (1958) who found that the 2-day larvae of carp are drastically different from 6-day-old fish in their sensitivity to endrin. They found that the 96-hour TL_m value for carp decreased from 10.7 p.p.m. of endrin at 2 days of age to 0.046 p.p.m. at 6 days of age. This represents a change of toxicity in the order of 250 times. It is quite probable that during this 4-day period the mouth became functional and the larvae began to drink water containing endrin.

It is also possible that endrin is excreted by the intestine and so appeared in the distillate of the digestive tract. Since the intestine was not emptied daily, there could have been an accumulation of excreted endrin in the lumen.

The positive values for the brain in test 5, and for fat in tests 2 and 5, are in agreement with the known storage of chlorinated-hydrocarbon insecticides in those tissues (Sherman and Rosenberg, 1953; Kunze and Laug, 1953; Treon, Cleveland, and Cappel, 1955; and Bann et al., 1956).

Nothing has been said up to this point concerning the absolute concentration present in the carp tissues. One must be very cautious in estimating concentrations based on bioassay, especially with a small sample. However, Klein et al. (1956) and Davidow and Sabatino (1954) have predicted concentrations on the basis of two goldfish in 250 ml. of distillate. They found the goldfish to give as reliable results as assays using 100 flies.

Perhaps an idea of the order of magnitude can be obtained from the available data. Only in test 5 (table 11) is the time until convulsions in the extract close enough to that in a known solution to warrant an actual estimate of the concentration of the extract. The time until convulsions in heart and spleen extract was the same as in 10 p.p.b. of endrin. If we assume 1 ml. of water to weigh 1 gram, there must have been a total of approximately 2.5 micrograms of endrin present. Since the tissue weight was 6.2 grams, this represents a concentration in the tissue of approximately 400 p.p.b. The concentration in the kidney would be slightly less, as would the concentration in the brain-spinal cord tissue. This represents a level 160 times more concentrated in the tissue than in the water in which the fish was living. The evidence indicates that endrin is actively taken up by some process at some point or points in the body.

It appears that if these estimates are even approximately reliable, there would be little danger to mammals eating exposed fish. These fish, eaten in normal quantities, would contain less than a lethal dose of endrin. The hazard would be further reduced in the case of man, since he normally eats only the muscle tissue, which gave negative results in three out of four tests. Most cooking methods would also evaporate much of the endrin, although Hunt and Bischoff (1960) found that baking fish in foil did not remove DDD.

Muscle from a carp exposed to 2.5 p.p.b. endrin for 4 weeks was fed to two male mice for 3 weeks. Neither mouse showed weight loss or any symptoms of poisoning, indicating that endrin was not present in concentrations high enough to be damaging. Of course, little is known about the effects of very minute amounts consumed by several generations of animals, and it is not safe to draw a conclusion based on this small sample.

In summary, this experiment showed highest concentrations of endrin in the liver, heart-spleen, and kidney. These findings agree with work cited on page 4, in which experiments with rats chronically exposed to endrin revealed that these organs were the ones evidencing tissue damage or storage of endrin. The presence of endrin in the brain and fat in test 5 agrees with the work of other investigators who found that chlorinated hydrocarbons are stored in the brain and fat. However, the cited investigation on mammals did not show any damage or accumulated endrin in the intestine, whereas the carp intestine contained measurable amounts.

MODE OF ACTION OF ENDRIN ON ANIMALS

When a substance is as toxic to an animal as endrin is to fish, the question arises what the specific effect might be. The concentration toxic to fish is usually a few parts per billion, whereas the level toxic to insects is in the range of 100 or more parts per billion. It has been found also by Henderson, Pickering, and Tarzwell (1959) and Iyatomi et al. (1958) that the range of the $\mathrm{TL}_{\overline{m}}$ values among species of fishes tested is not over 5 parts per billion. Endrin's toxicity to mammals is also less than its toxicity to fish. Ely et al. (1957) reports 1.5 mg./kg. to be toxic to cattle, and Treon, Cleveland, and Cappel (1955) determined that 43.4 parts per million of endrin in food was toxic to rats.

Hartley and Brown (1955) reported that endrin had no effect on the cholinesterase level in the American cockroach. Morrison and Brown (1954) found that compounds related to endrin had no effect on the cytochrome oxidase level in the same animal. Nelson (1955) states that serum alkaline phosphatase levels were higher in test than in control rats fed 100, 50, 25, 5, and 1 p.p.m. of endrin in their food. (In human beings an increase in the serum alkaline phosphatase levels may indicate liver disturbances.—Hawk, Oser, and Summerson, 1954). All rats were hypersensitive to stimuli, and those fed 100, 50, and 25 p.p.m. endrin in the food had convulsive spasms. Roeder (1948) found that technical chlordane (related to endrin chemically) had no effect on synaptic transmission of impulses in the nerve preparation of *Periplaneta* and caused no neurologic disturbances in mammals.

Harvey and Brown (1951) stated that all insecticides which increase oxygen consumption are known to be nerve poisons. He used chlordane as an example of one which is not a nerve poison and which does not increase oxygen consumption significantly.

A clue to the possible mode of action was found during the testing in this investigation. The symptoms—convulsions and loss of equilibrium—indicate disturbances in the central nervous system. There was always an increased ventilation rate but, as was pointed out earlier, the oxygen consumption did not increase. In many of the young guppies which died in endrin, the skull burst and the brain protruded. This indicated pressure on the central nervous system. Such pressure would also stimulate the respiratory center, produce convulsions, and cause loss of equilibrium.

A condition called papilledema in humans, in which the optic disk swells as a result of pressure in the central nervous system, is used to detect diseases which cause an increase in pressure on the brain in man. The pressure is transmitted along the optic nerve, collapses the blood vessels, and causes the optic disk to swell because of the blocked circulation.

I attempted to find papilledema in fish by fixing and measuring optic disks of normal fish and of fish which had died in 10 p.p.b. endrin. Four fish were placed in 10 p.p.b. endrin and four of similar size were placed in tap water. As the fish in endrin lost equilibrium they were put into A. F. A. fixing solution. When the fourth test fish was fixed, the control fish were fixed also. After 12 hours in the fixative, the eyes were removed and imbedded in paraffin. Serial cross-sections 15 microns thick were made and stained with eosin. Measurements were made with an ocular micrometer.

The results of this experiment are shown in table 12. Two measurements were taken on each eye; the minimum thickness of the two middle sections was measured. No significant difference was found between the treated and the control groups. It is possible that shrinkage due to fixation obliterated any swelling which may have been present.

TABLE 12.—*Optic-disk measurements of the bluntnose minnow*

[Two sections of each disk were measured. Numbers represent ocular-scale divisions in which 1 division equals 6.8 microns]

Normal fish		Fish in 10 p.p.b. endrin	
Left eye	Right eye	Left eye	Right eye
22	28	33	24
20	26	31	25
40	28	30	20
45	27	33	25
24	20	22	24
21	19	21	20
28	25	35	25
31	23	30	25
$\bar{m}$ length of fish=48 mm. standard length $\bar{m}$ thickness of optic disk= 26.7 ocular divisions		$\bar{m}$ length of fish=46.8 mm. standard length $\bar{m}$ thickness of optic disk= 26.4 ocular divisions	

The carp used in the investigation of the entry and distribution of endrin in the body were examined with an ophthalmoscope while convulsions were in progress, but it was very difficult to see into the eye and I could not tell anything about the optic disk. Carp were difficult to hold still, and the examination was unsatisfactory.

When fish showing symptoms of poisoning are placed in pure water, they usually recover rapidly. In one instance, 10 bluntnose minnows were placed into a continuously renewed solution of 0.3 p.p.b. When five fish had died and the other five were having convulsions, the flow was stopped. Two days later approximately half of the 9 gallons in the test chamber were replaced with tap water. These five fish went through the symptoms of endrin poisoning in a reverse order, and at the date of this writing, 3 months later, appear normal and healthy. This type of reaction indicates that endrin does not cause irreversible damage.

The available information concerning the mode of action of endrin on animals does not permit any conclusion to be drawn about the specific effects of endrin. This present investigation furnishes little new information other than that endrin does not appear to increase oxygen consumption, nor does it appear to cause irreversible damage as a result of short exposures.

SUMMARY

Investigations of the chronic toxicity of endrin to bluntnose minnows, *Pimephales notatus*, and guppies, *Lebistes reticulatus*, were conducted for periods of 10 and 6 months, respectively. Continuously renewed solutions were employed.

Approximately 35 percent of the bluntnose minnows survived for extended periods in 0.5 p.p.b. endrin in water, and approximately 50 percent survived 0.4 p.p.b. All bluntnose minnows placed in 0.1 p.p.b. lived for extended periods; all died in 0.75 and 0.9 p.p.b. within a 29-day period.

Symptoms of endrin poisoning in the bluntnose minnows were characteristic of disturbances in the central nervous system: hyperactivity, increased ventilation rate, convulsions, loss of equilibrium, and death. The symptoms lasted for longer periods at lower concentrations. Some fish were in a state of hyperactivity and increased ventilation rate for as long as 2 months and then returned to normal condition while still in endrin. Mean daily growth rates in 0.5, 0.4, 0.1, and 0 p.p.b. endrin were 0.049, 0.047, 0.102 and 0.069 mm., respectively. The toxic levels for chronic exposures were not greatly different from those toxic in acute exposures. Fish in 0.5 and 0.4 p.p.b. exhibited much more activity than those in 0.1 p.p.b. and in the control aquariums. The gonads in both sexes in all test chambers showed increased activity and appeared to be ripening about as rapidly as those of fish taken from the stream. Histological examination showed fatty vacuolation of the liver cells in many of the fish, including control fish. Such vacuolation is thought to be due to the high fat content of the diet fed. No tissue damage was found in the brain, heart, kidney, or spleen.

A chronic-toxicity test on guppies was conducted simultaneously with the bluntnose minnow test. Because of a disease of the kidneys which was apparently unrelated to the presence of endrin, tests on the guppies were discontinued after 6

months. The effects of endrin on the guppies were essentially the same as those on the bluntnose minnows.

Pregnant female guppies, with one exception, never had more than one litter of young after being placed in 0.75 and 0.5 p.p.b. endrin in water. In one case, a second litter of 12 was produced and included 6 young with large yolk sacs, presumably born prematurely. All young born from females in endrin, including the premature ones, survived about as long as guppies born from parents not in endrin. No sexual difference in survival was found. Adults were usually more tolerant than young.

One major difference in behavior was found during the period of poisoning; guppies went to the bottom and bluntnose minnows went to the surface. The normal resting and feeding positions of these species are just the reverse.

In acute-toxicity testing of bluntnose minnows, fish with a mean standard length of 59.8 mm. withstood 0.2 p.p.b. more endrin in 96 hours than did fish of mean standard lengths of 29.8 and 40.6 mm. The fish used in the chronic-toxicity test (collected in March) proved to be more tolerant to endrin than those used in the acute-toxicity tests (collected in the winter). This may have been due to one or more of the following: (1) the stage of the reproductive cycle, (2) parasitism, (3) seasonal temperatures and related physiological conditions, (4) crowded conditions in the stream resulting in less hardy fish.

Endrin had no apparent effect on oxygen consumption of fed and nonfed fish of either sex. Endrin did not seem to affect the ability of fish to swim against a current of water.

Evidence was found which indicates that endrin enters the bodies of carp through the intestines. It probably is carried by the blood to other parts of the body. The liver, intestine, spleen, and kidney of carp contained the highest concentrations of endrin in 2- to 5-day exposures. Blood, fat, and brain tissue also contained measurable amounts after a 28-day exposure. All concentrations in carp tissues were based on a fish bioassay of distillates collected by steam distillation.

The bursting of the skull and subsequent protrusion of the brain of young guppies which died in various concentrations of endrin indicated that endrin may cause pressure in the central nervous system.

CONCLUSIONS

Endrin does not appear to be cumulative in its effect on fish subjected to chronic exposures. There was a narrow range from 0.4 to 0.5 p.p.b. in which fish showed some loss of weight and greatly increased activity. No effect was found below this concentration. Although no reproduction occurred in the bluntnose minnow, the gonadal development indicated that reproduction might have taken place if the test had been continued. Concentrations as low as 0.5 p.p.b. appeared to prevent reproduction in guppies.

It appears that the increase in activity caused by endrin in concentrations of 0.5 and 0.4 p.p.b. might be of greatest significance in natural populations through disruption of spawning behavior and displacement from their normal habitat niche.

On the basis of the data, endrin appears to enter the body through the intestine as a result of the fish's drinking water. Very young fish are resistant to endrin, probably because their mouths are not functional and the endrin does not get into the intestine.

There is evidence of a slight accumulation of endrin in carp tissue during a 28-day exposure. The low concentration found in the muscle tissue would probably not be harmful to human beings eating exposed fish. Cooking would also reduce the concentration.

It appears on the basis of this investigation that endrin would not harm fish seriously so long as a concentration which is not lethal in 30 days is not exceeded. Endrin is extremely toxic to fish, and application rates would have to be very low. However, if endrin will effectively control pests at an application rate which would be safe for fish, its use would be preferable to that of a compound less toxic to fish but with greater cumulative effects.

There is a very serious need for chronic-toxicity studies of the effects of many insecticides on all aquatic forms. Not one such study was found in the literature. Investigations are needed to determine which organisms in the aquatic food chains are most sensitive to various pollutants. More work should be done to determine the residual life of these pollutants in the bottom sediments and in the tissues of animals. Until this information is known, the danger involved in continuous use of insecticides cannot be determined. There is little knowledge at the present time to indicate what danger there is to human beings, either directly or indirectly, from continuous use of insecticides.

Finally, it will be necessary to gain a better understanding of normal fish physiology before the true danger of insecticides to fish can be properly assessed.

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Appendix—RAW DATA FROM TESTS

APPENDIX TABLE 1.—*Results of acute-toxicity testing on bluntnose minnows*

["Juvenile" indicates fish too young to sex easily; "A" indicates fish living at end of test]

	Standard length (mm.)	Number of days lived
Test S–9:		
0.8 p.p.b. endrin:		
Juvenile	26	1
Do	27	1
Do	30	1
Do	31	1
Do	32	1
0.6 p.p.b. endrin:		
Juvenile	33	1
Do	24	1
Do	33	1
Do	34	1
Do	29	1
0.4 p.p.b. endrin:		
Juvenile	25	2
Do	29	2
Do	30	2
Do	28	3
Do	34	3
0.2 p.p.b. endrin:		
Juvenile	32	3
Do	22	A
Do	28	A
Do	28	A
Do	31	A
Mean size	29.3	
Test S–10:		
0.8 p.p.b. endrin:		
Juvenile	27	1
Do	28	1
Do	34	1
Do	26	2
Do	34	2
0.6 p.p.b. endrin:		
Juvenile	26	1
Do	26	2
Do	27	2
Do	32	2
Do	31	3
0.4 p.p.b. endrin:		
Juvenile	26	3
Do	27	3
Do	28	3
Do	31	3
Do	32	3
0.2 p.p.b. endrin:		
Juvenile	27	A
Do	28	A
Do	30	A
Do	33	A
Do	34	A
Mean size	29.4	
Test S–11:		
0.8 p.p.b. endrin:		
Male	65	2
Do	66	2
Female	60	2
Male	58	2
Do	81	4
0.6 p.p.b. endrin:		
Male	71	2
Do	70	3
Do	64	3
Do	59	4
Do	70	A
0.4 p.p.b. endrin:		
Male	68	A
Do	70	A
Do	60	A
Female	56	A
Male	57	A
0.2 p.p.b. endrin:		
Male	65	A
Do	57	A
Female	59	A
Do	56	A
Do	55	A
Mean size	63.4	

	Standard length (mm.)	Number of days lived
Test S–12:		
0.8 p.p.b. endrin:		
Male	37	1
Do	37	1
Female	39	1
Do	36	1
Do	33	1
0.6 p.p.b. endrin:		
Female	35	1
Do	38	1
Do	41	2
Do	41	2
Male	36	2
0.4 p.p.b. endrin:		
Male	35	2
Female	39	2
Do	40	2
Do	38	A
Do	38	A
0.2 p.p.b. endrin:		
Male	30	A
Female	31	A
Male	40	A
Female	43	A
Do	43	A
Mean size	37.5	
Test S–13:		
0.5 p.p.b. endrin:		
Female	45	2
Male	46	2
Female	42	2
Male	40	2
Female	37	2
0.4 p.p.b. endrin:		
Female	35	2
Male	40	2
Do	39	2
Female	33	2
Male	39	3
0.3 p.p.b. endrin:		
Male	49	3
Female	36	3
Male	39	3
Female	40	4
Do	39	4
0.2 p.p.b. endrin:		
Female	42	A
Do	42	A
Male	47	A
Female	41	A
Male	39	A
Mean size	41.0	
Test S–14:		
0.5 p.p.b. endrin:		
Male	66	3
Female	59	3
Do	56	3
Male	55	3
Do	54	3
0.4 p.p.b. endcrin:		
Female	51	4
Male	75	A
Female	53	A
Male	56	A
Female	49	A
0.3 p.p.b. endcrin:		
Male	71	A
Female	63	A
Male	59	A
Female	57	A
Do	51	A
0.2 p.p.b. endrin:		
Female	53	A
Do	53	A
Male	61	A
Female	55	A
Do	52	A
Mean size	57.1	

Appendix table 1.—*Results of acute-toxicity testing on bluntnose minnows*—Continued

	Standard length (mm.)	Number of days lived
Test S-15:		
0.5 p.p.b. endrin:		
Juvenile	32	2
Do	27	2
Do	30	2
Do	32	2
Do	33	2
0.4 p.p.b. endrin:		
Juvenile	27	4
Do	30	4
Do	33	4
Do	31	A
Do	30	A
0.3 p.p.b. endrin:		
Juvenile	30	3
Do	33	3
Do	26	4
Do	31	A
Do	32	A
0.2 p.p.b. endrin:		
Juvenile	24	A
Do	37	A
Do	32	A
Do	34	A
Do	35	A
Mean size	30.7	
Test S-16:		
0.5 p.p.b. endrin:		
Female	53	2
Do	41	2
Do	49	3
Do	36	3
Do	38	3
0.4 p.p.b. endrin:		
Female	50	3
Male	40	4
Female	42	4
Do	41	4
Do	52	A
Test S-16—Continued		
0.3 p.p.b. endrin:		
Male	41	4
Do	44	4
Female	43	4
Do	40	A
Do	52	A
0.2 p.p.b. endrin:		
Male	40	A
Female	36	A
Do	41	A
Male	39	A
Do	50	A
Mean size	43.2	
Test S-17:		
0.5 p.p.b. endrin:		
Male	57	3
Do	62	3
Do	64	3
Do	60	4
Do	65	A
0.4 p.p.b. endrin:		
Male	69	A
Do	62	A
Female	58	A
Unrecorded		A
0.3 p.p.b. endrin:		
Female	53	A
Do	55	A
Do	57	A
Male	65	A
Female	54	A
0.2 p.p.b. endrin:		
Female	55	A
Male	56	A
Do	65	A
Female	55	A
Do	52	A
Mean size	58.9	

Appendix table 2.—*Results of tests on oxygen consumption in the bluntnose minnow*

[Consumption is expressed in ml. per gram per hour. All testing was done at temperatures between 23° and 24° C.]

Test O-1: A 0.7-gram female, 38 mm. standard length. Consumption was measured at 7 a.m., 12 hours after the fish was fed.

Normal consumption	*Consumption in 0.4 p.p.b. endrin*
0.366	0.674
.320	.469
.320	.435
.366	.366
.366	.213
.352	.366
	.366
Mean .348	.366
	.160
	.320
	.275
	.310
	.354
	.443
	Test discontinued
	Mean .366

Test O-2: A 4.2-gram male, 70 mm. standard lengh. Consumption was measured daily at 7 a.m., 12 hours after feeding.

Normal consumption	*Consumption in 0.5 p.p.b. endrin*
0.210	0.184
.215	.230
.223	.267
.209	.329
.244	Fish died
.220	
.279	Mean .253
.215	
.215	
.188	
Mean .222	

APPENDIX TABLE 2.—*Results of tests on oxygen consumption in the bluntnose minnow*—Continued

Test O–4: A 5.0-gram male, 73 mm. standard length. Consumption was measured at 7 a.m. daily, 24 hours after feeding.

Normal consumption	*Consumption in 0.4 p.p.b. endrin*
0. 135	0. 285
. 181	. 219
. 198	. 305
. 221	. 316
. 272	Fish died
. 266	
. 186	
. 218	
. 280	
. 175	
. 272	
. 299	
. 169	
. 249	
. 327	
. 266	
. 270	
. 150	
Mean . 230	Mean . 281

Test O–5: A 2.0-gram female, 56 mm. standard length. Consumption was measured daily at 7 a.m. The fish was not fed.

Normal consumption	*Consumption in 0.4 p.p.b. endrin*
0. 181	0. 073
. 133	. 104
. 182	. 151
. 074	Fish died
. 044	
. 236	
. 042	
. 021	
. 127	
. 094	
. 106	
. 130	
. 134	
. 080	
Mean . 113	Mean . 109

Test O–6: A 2.6-gram female, 60 mm. standard length. Consumption was measured twice daily at 7 a.m. and at 6 p.m. The fish was not fed.

Normal consumption	*Consumption in 0.3 p.p.b. endrin*
0. 176	0. 217
. 185	. 209
. 193	. 217
	. 217
Mean . 185	. 194
	. 180
	. 173
	. 184
	. 184
	. 147
	. 137
	Mean 0. 187

Test O–7: A 3.0-gram male, 61 mm. standard length. Consumption was measured twice daily, at 7 a.m. and 6 p.m. The fish was not fed.

Normal consumption	*Consumption in 0.5 p.p.b. endrin*
0. 181	0. 219
. 191	. 207
. 228	. 184
. 197	. 233
. 224	Fish died
Mean . 204	Mean . 211

Test O–8: A 3.0-gram male, 61 mm. standard length. Consumption was measured twice daily, at 7 a.m. and 6 p.m. Fish was not fed and was starved seven days before the test was begun.

Normal consumption	*Consumption in 0.5 p.p.b. endrin*
0. 142	0. 116
. 132	. 136
. 107	. 144
. 130	. 164
	. 241
Mean . 128	Fish died
	Mean . 160

PREDICTING YEAR-CLASS ABUNDANCE OF YELLOWSTONE LAKE CUTTHROAT TROUT

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RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

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Norman G. Benson
Fishery Research Biologists

RESEARCH REPORT 59

UNITED STATES DEPARTMENT OF THE INTERIOR
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FISH AND WILDLIFE SERVICE
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ABSTRACT

Fluctuations in strength of year classes from 1945 to 1956 of Yellowstone Lake cutthroat from Pelican and Chipmunk Creeks are compared with the parental stock and several climatically influenced factors of the environment. Variations in year-class strength in the two tributaries were highly correlated with fluctuations in lake water levels. Strong year classes occurred in years of low water. Female spawner escapement, timing of the runs, and summer air temperatures were not significant factors.

A formula based on water levels is presented for predicting year-class strength in Pelican Creek and in the Fishing Bridge area fishery.

Stocking of fry in years of high water is suggested as a means of supplementing natural production. A method of forecasting lake water levels several months in advance of their occurrence is discussed.

PREDICTING YEAR-CLASS ABUNDANCE OF YELLOWSTONE LAKE CUTTHROAT TROUT

By Ross V. Bulkley and Norman G. Benson, ***Fishery Research Biologists***

Rocky Mountain Sport Fishery Investigations, Logan, Utah

Large fluctuations occur in year-class strength of cutthroat trout, *Salmo clarki lewisi*, in Yellowstone Lake, Wyoming. The ability to predict the occurrence of these fluctuations is of great value in managing the fishery. This paper discusses five factors suspected of controlling year-class strength in Pelican and Chipmunk Creeks, two large tributaries of Yellowstone Lake, and the use of these factors to predict future recruitment to the fishery.

Pelican Creek is one of the major spawning streams in the north end of Yellowstone Lake (fig. 1). Terminal water flow in August is around 40 cubic feet per second, but the flow is several times as large during the spring high-water period. The Pelican Creek drainage contains approximately 100 miles of stream available for spawning. Adult fish move into the stream during May, June, and early July for spawning, and return to the lake after spawning. A few spawners overwinter in the stream. Many fry remain in the stream during their first winter and move down to the lake in their second and third years of life. Recruitment to the spawning runs occurs during the fourth and fifth years of life.

Chipmunk Creek enters Yellowstone Lake at the lower end of the south arm. Terminal stream flow during August is around 25 cubic feet per second. The spring runoff is more gradual than Pelican Creek, and is relatively free of debris. Spawning runs in Chipmunk Creek are bimodal, commencing in May and extending to the end of July (Cope, 1957b). Most spawning occurs in the lower 8 miles of the stream, and the majority of the fry move downstream to the lake during their first summer. Initial spawners in this stream are fish of age groups III, IV, and V; some fish from Chipmunk Creek do not mature until their sixth year of life (Bulkley, 1961). In both streams, larger and older spawners are most abundant in the early part of each spawning run, and smaller spawners are most abundant in the latter part of each run.

Counts of spawners and collections of scales were made from Pelican and Chipmunk Creeks spawning runs from 1949 to 1960. Scale samples and measurements were taken from 10 percent of each run. Data from upstream traps near the mouth of each stream provided information on the time and size of the spawning migrations. Scale samples from the fishery were obtained periodically during the summer from 1950 to 1960 at Fishing Bridge and West Thumb docks. Fish from Pelican Creek are commonly caught in the Fishing Bridge area, as this stream enters the lake 1 mile from the Fishing Bridge campground (Ball and Cope, 1961). Chipmunk Creek fish contribute to the West Thumb fishery, but the percentage of the West Thumb catch that comes from fish produced in that stream is not known.

Scales were aged according to criteria established by Laakso and Cope (1956) for identification of fish which fail to form the initial annulus on their scales. Cutthroat

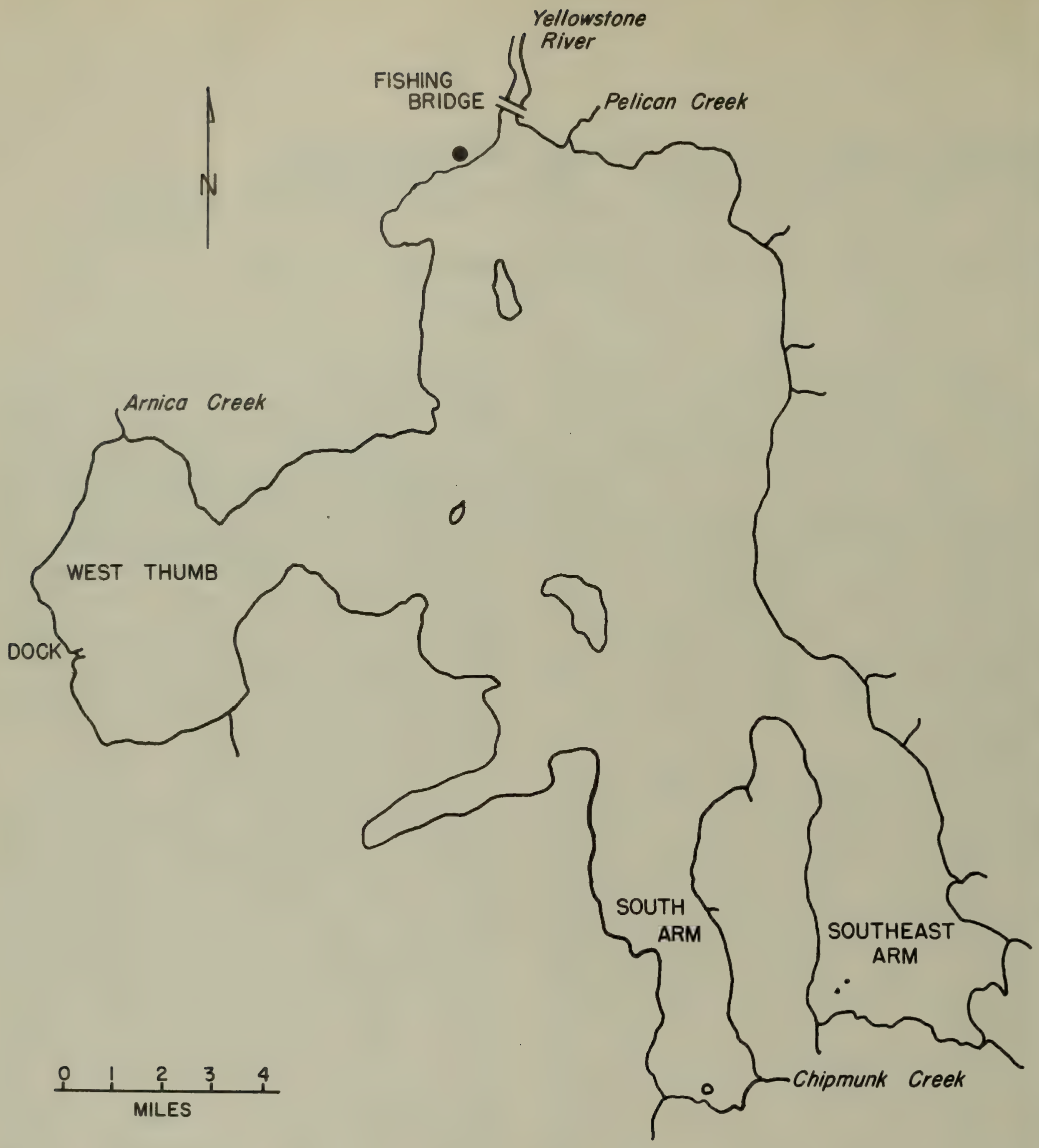

Figure 1.—Yellowstone Lake showing location of two study streams. The area where water levels and air temperature were recorded is indicated by closed circle near Fishing Bridge.

trout with retarded scale development are relatively common in Yellowstone Lake.

Daily gage heights of Yellowstone Lake at the Lake Hotel were obtained from reports of the U.S. Geological Survey. Daily air temperatures at the Lake Ranger Station were obtained from U.S. Weather Bureau reports. Data used in this paper on the lake water levels and on air temperatures are summarized in appendix A.

Drs. W. E. Ricker, George A. Rounsefell Oliver B. Cope, and Mr. Bernard E. Skud critically reviewed the manuscript and offered several excellent suggestions for its improvement. Messrs. Ashton R. Codd and Phillip E. Farnes of the Soil Conservation Service provided the forecasts of Yellowstone Lake water levels.

YEAR-CLASS STRENGTH

Estimates of year-class strength were determined from the number of fish of both sexes assumed to be initial spawners returning to the parent stream from each year class. Fish of age groups III and IV were used for Pelican Creek; fish of age groups III, IV, and V were used for Chipmunk Creek. A few spawners are undoubtedly counted twice by this method, but spawning mortality is sufficiently high that only a small percentage survive to return as repeat spawners (Ball and Cope, 1961). Most of the spawners which do return require 2 years to recover and come back as alternate spawners. Recruitment of fish to the spawning runs was considered to be a good index of relative year-class strength, as the homing instinct is strong and few fish spawn in streams other than their parent stream (Ball, 1955). The lake fishery is concentrated on the same age groups as found in the spawning runs, and recruitment to the fishery of prespawners was independent of the size of the catch prior to the 1956 year class in Pelican Creek and to the 1955 year class in Chipmunk Creek.[1] Thus, variations in the catch did not seriously alter estimates of year-class strength based on numbers of initial spawners. This method could not be used for Pelican Creek from 1945 to 1947 because 500,000 fry were planted in that stream each year. No fry were planted in Chipmunk Creek after 1944.

Pelican Creek produced large year classes in 1949, 1953, and 1955 (fig. 2). Chipmunk Creek estimates (no data available for 1956) indicate that 1945, 1946, and 1953 were large year classes and the 1951 year class was extremely small. Year-class strength of the two streams is obviously unrelated in some years.

FACTORS COMPARED WITH YEAR-CLASS STRENGTH FLUCTUATIONS

Benson (1960) showed that receding water levels and cloudy weather partially controlled the downstream migrations of immature cutthroat trout in Arnica Creek, a small tributary to Yellowstone Lake, but there was no relation between this migration of immatures and the number of adults returning. As accurate data were not available on stream-level fluctuations and light intensity at Pelican and Chipmunk Creeks, these factors could not be tested in the present study. Factors tested for association with year-class strength in Pelican and Chipmunk Creeks included female spawner escapement, timing of the spawning runs, air temperature, and lake water levels. Pelican Creek anaylses are presented first, with field data for both streams. Results of Chipmunk Creek analyses are presented later for clarity.

Female escapement

The number of spawners producing a year class was one factor suspected of controlling year-class strength A large egg potential under certain conditions could be expected to produce an abundant year class. Escapement of female spawners was compared with estimates of year-class strength to determine whether size of parental stock was controlling year-class strength in the two study streams. Numbers of female spawners were used because the size of male escapement was not recorded before 1954. Escapement of female spawners in Pelican and Chipmunk Creeks fluctuated widely from 1945 to 1956 (table 1). Much of this fluctuation

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[1] "Equilibrium yield and management of cutthroat trout in Yellowstone Lake," by Norman G. Benson and Ross V. Bulkley. Manuscript in preparation.

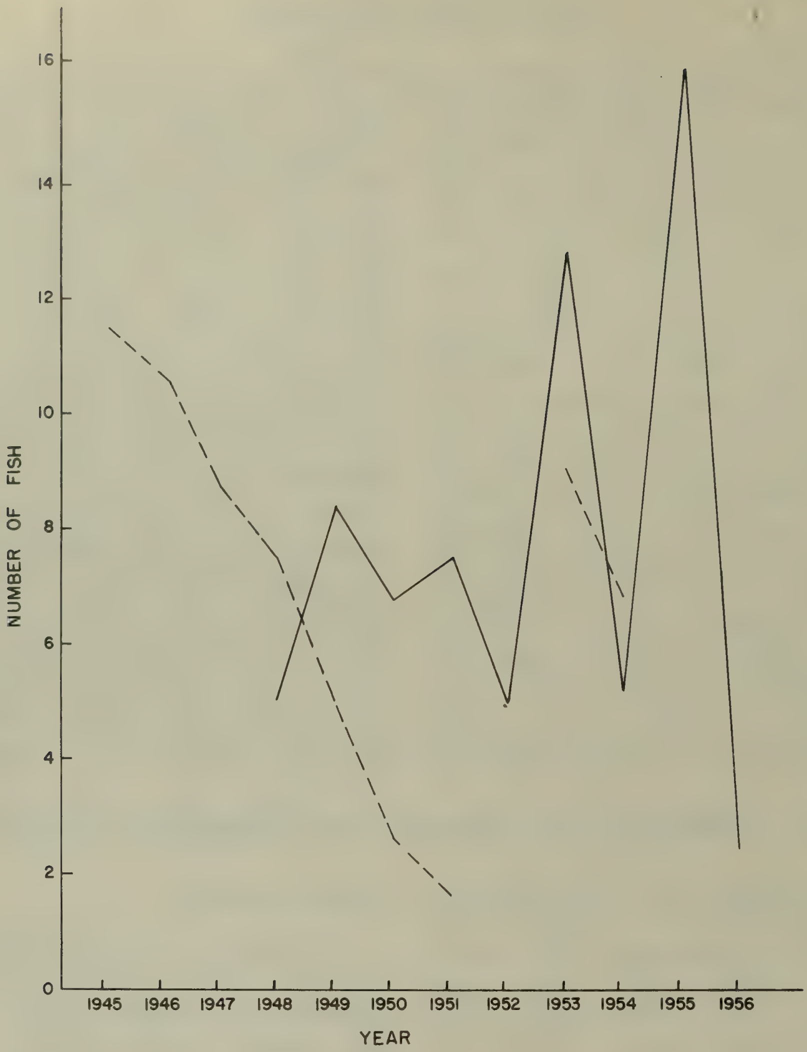

FIGURE 2.—Year-class strength for Pelican (solid line) and Chipmunk Creeks (broken line)—expressed in thousands of fish.

was caused by fish-hatchery operations at Yellowstone Lake, which continued until 1953. Eggs for the hatchery were obtained from wild fish as they ascended the tributaries to spawn. The number of females released for natural spawning depended on the size of the spawning runs and the number of eggs needed by the hatchery each year. As all males not needed for spawn-taking were allowed to continue upstream, there is little reason to suspect that a shortage of males for natural spawning ever occurred. After 1953 all spawners were allowed to spawn naturally.

Analysis of the relation between Pelican Creek female escapement and number of progeny returning to spawn from the year class did not show a significant correlation ($r=0.480$, $d.f.=7$) although the plotted data suggest that a rather strong positive correlation existed (fig. 3). Low female escapement tended to have low production, and high escapement to have high production. The 1949 data

TABLE 1.—*Size of female escapement in Pelican and Chipmunk Creeks and recruitment to future runs from each year class*

Year-class	Female escapement		Recruitment	
	Pelican Creek	Chipmunk Creek	Pelican Creek [1]	Chipmunk Creek [2]
1945	----------	2,331	----------	11,524
1946	----------	6,350	----------	10,665
1947	----------	841	----------	8,838
1948	4,418	2,449	5,032	7,500
1949	14,591	2,650	8,377	5,286
1950	3,381	2,628	6,755	2,729
1951	1,969	[3] 2,398	7,569	----------
1952	613	1,695	4,985	----------
1953	7,475	2,711	12,863	9,184
1954	6,438	3,237	5,267	6,921
1955	9,030	2,678	16,023	----------
1956	[3] 4,166	----------	2,503	----------

[1] Total fish of age III and IV returning as first spawners from year class.

[2] Total fish of age III, IV, and V returning as first spawners.

[3] Complete count not obtained.

suggest that production falls off as female spawner numbers approach 14,000. The "Ricker Curve" (Ricker, 1958, p. 239), which passes through the origin and falls off asymptotically at high stock levels, seemed to describe the Pelican Creek population but did not reduce the residual variance. The free-hand curve illustrated was fitted to the data with the aid of moving averages of three and may be closer to the true relation. This curve suggests that increased production can be obtained by increasing female escapement up to approximately 9,000 females. Sheppard and Withler (1958) described a similar reproduction curve for sockeye salmon. Assuming a continuation of the average sex ratio of Pelican Creek runs of 1.64 females per male (Ball and Cope, 1961), 9,000 females would represent a total spawning run of approximately 14,500 fish. The broken diagonal line in figure 3 is based on the average sex ratio and indicates the level at which replacement occurs. Each female must produce on the average 1.61 adult progeny for the population to maintain the same density. Thus escapement to the right of the diagonal failed to replace itself (1948, 1949, 1954, and 1956) and escapement to the left more than replaced itself.

Data presented later on water levels and the low correlation between female escapement and progeny suggest that escapement is a secondary factor in controlling production within the range of spawners given above. Benson (1960) and Ball and Cope (1961) did not find any correlation between number of spawners and number of progeny produced.

Time of spawning

Timing of spawning runs is frequently considered a possible control of production. Exceedingly early or late segments of the spawning runs are often less successful than the middle part owing to adverse environment (Rounsefell, 1958). Spawning runs in Pelican and Chipmunk Creeks usually start about the same time each year, although there is considerable variation in the time in which one-half of the run passes upstream in different years. The date by which 50 percent of the run entered the upstream trap was compared with the relative strength of the year class which the run produced, to measure the effect of these variations in timing (table 2).

TABLE 2.—*Date by which 50 percent of spawning run entered upstream traps at Pelican and Chipmunk Creeks*

[Expressed as the number of days after May 28]

Year	Pelican Creek	Chipmunk Creek
1945	----------	25
1946	1	4
1947	4	9
1948	----------	12
1949	2	27
1950	21	41
1951	9	----------
1952	11	----------
1953	18	13
1954	10	38
1955	11	----------

Timing of the run apparently did not influence production in Pelican Creek to any extent as a correlation coefficient of 0.115 (*d.f.*=5) was far from significant.

Air temperature

Air temperatures were considered a factor controlling production under the

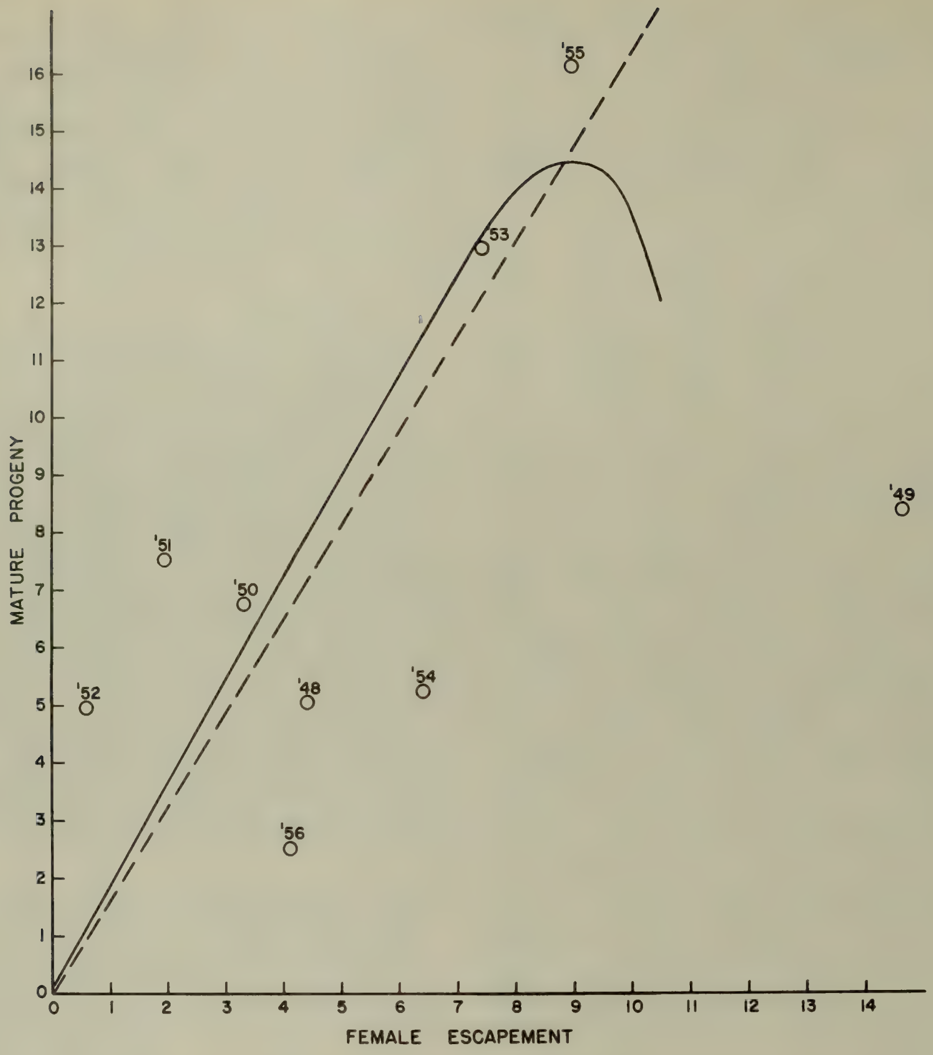

FIGURE 3.—Estimated reproduction curve for Pelican Creek, based on data from 1948 to 1956—expressed in thousands of fish. The broken line indicates the level at which replacement of parental stock occurs.

assumption that high spring temperatures would cause rapid snow melting with ensuing flooding of redds, and that high summer temperatures could conceivably cause fry mortality from exessive water temperatures. The assumptions are not unreasonable, as Pelican Creek has many thermal springs (Cope, 1957a), and warm weather during the spawning migration frequently causes flooding at the Pelican Creek trap and occasionally at the Chipmunk Creek trap. Correlation between year-class strength and mean air temperatures during the main spawning period (June–July) was not significant for Pelican Creek ($r=0.325$, $d.f.=7$).

Temperature data not presented in this paper indicate that water temperatures were highest during July in Pelican Creek, which is the major incubation period in that stream. Correlation between mean air temperature during July and year-class strength was also not significant ($r=0.049$). The influence of air temperature is probably obscured by more prominent factors such as stream flow, which is partially controlled by air temperatures during the spawning period.

Water levels

Excessive stream flow can affect production of salmonids by decreasing the efficiency of egg deposition, by erosion or siltation of the redds, or by actual destruction of fry (Gangmark and Bakkala, 1960). Low water levels may limit spawning in the most favorable areas or cause exposure of redds. As stream flow data were not available, lake water levels were used as an index of stream flow into the lake. Yellowstone Lake is fed by a large tributary system, and fluctuations in lake level should be representative of tributary flow. In the summer of 1960 the trend in lake levels was similar to Pelican Creek water levels, but lagged approximately a week behind the stream. No data were available to compare Chipmunk Creek stream flow with lake levels.

The nature of the spring runoff in Pelican Creek suggests that stream flow could easily influence trout production. Pelican Creek is noted for its high spring runoff, which commonly flows over the trap and necessitates continual cleaning of the weir. In high-water years large trees are often flushed downstream and deposited against the weir. The Pelican Creek spawning migration usually commences in earnest about May 18, and young-of-the-year begin emerging from the gravel in the middle of July. Allowing 1 week for the fish to reach the spawning sites, the incubation period starts about May 25 and ends by July 31. The wide range in the time by which 50 percent of the run migrated upstream suggests that the incubation period varies slightly from year to year and that more accurate comparisons with year-class strength could be made if these variations were considered. The spawning runs usually start and end about the same time each year, even though variations occur in the time by which one-half of the run passes upstream. The part of the run which produces the year class is also not known. Thus, lake water levels from June 1 to July 31 were used in Pelican Creek comparisons, as this period covered most of the spawning and incubation in all years.

Pelican Creek data suggested a curvilinear relation between mean water levels and year-class strength from 1948 to 1956, and estimates of year-class strength were transformed to base–10 logarithms for comparison (fig. 4). Approximately 91 percent of the variation in production in Pelican Creek was associated with mean lake water levels during June and July ($r=-0.955$, significant at the 1-percent level). Assuming a linear relation between water levels and year-class-strength, correlation was only slightly lower ($r=-0.904$) and was still highly significant. Production was highest at low water levels and decreased as water level increased. A negative relation between water levels and production is uncommon, although Vernon (1958) found such a relation in pink salmon of the Fraser River. Poor production in flood years is to be expected, but drought years usually have low production also (Rounsefell, 1958; Wickett, 1958). Water levels in Pelican Creek apparently never became sufficiently low during the study period to be detrimental to production.

Differences between mean June and mean July water levels with the sign ignored were compared with year-class estimates for Pelican Creek to determine whether fluctuations in water level within the spawning period were influencing year-class strength. The correlation was not significant ($r=-0.278$, $d.f.=7$). Mean stream flow during spawning and incuba-

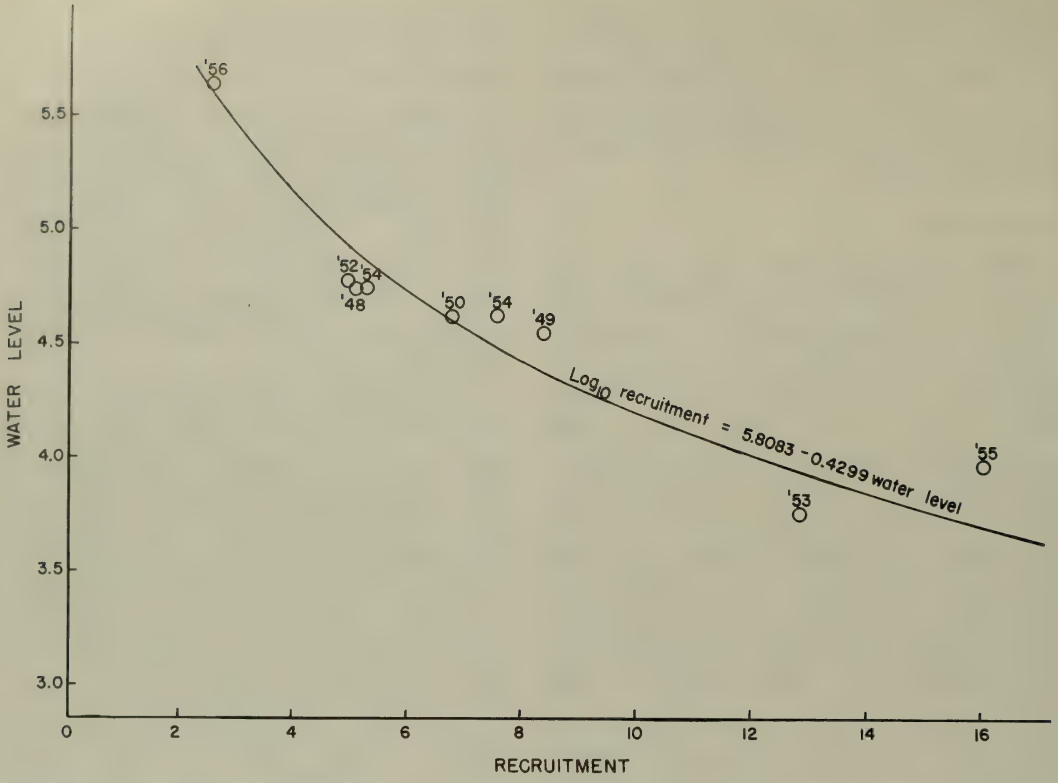

FIGURE 4.—Mean June-July water levels (feet) of Yellowstone Lake in relation to recruitment to the Pelican Creek spawning runs from each year class—expressed in thousands of fish.

tion was apparently the major controlling factor rather than the degree of fluctuation about the mean flow.

Combined effects

More variation in year-class strength could be explained when several factors were compared simultaneously. Frequently interaction between two factors masks individual effects on production. The effects of water levels, escapement, temperature, and timing of the runs on production were further analyzed by multiple regression analysis to determine whether two or more variables were interacting.

Data from 1948 to 1956 on female escapement, mean June-July water levels, and differences in mean June and mean July water levels were analyzed simultaneously with Pelican Creek year-class strength. As air temperature was believed to be influencing water conditions, it was not included in the analysis. Data on timing of the runs were not available for some years, and that variable was not included. Multiple correlation between year-class strength and escapement, water levels, and water fluctuations was significant at the 5-percent level ($R=0.925$, $d.f.=5$). The three factors were associated with approximately 77 percent of the variation (R_A^2) in Pelican Creek production.

When estimates of year-class strength were transformed to base-10 logarithms and were then correlated with escapement, mean water levels and water-level fluctuations, the correlation was significant at the 1-percent level ($R=0.980$)

and the amount of the variance related to these factors increased to 93.8 percent. Elimination of escapement from the multiple regression, and comparison of year-class strength with water levels and water fluctuations, reduced the correlation to 0.978, but the amount of variation in year-class strength (R_A^2) associated with these two measurements of water condition was 94.3 percent. This correlation was also significant at the 1-percent level.

A series of residuals (Ricker, 1958) was used to determine whether timing of the runs could account for the remaining variations in year-class strength after the effects on year-class strength of mean water level and water-level fluctuations were removed. The use of residuals was considered legitimate, as the effect of water levels on production has been reported frequently in the literature, and the concurrent fluctuations in water level and production in Pelican Creek strongly suggest that a causal relation exists. The residuals are the positive and negative deviations from the regression line which expresses the relation between log-10 year-class strength, mean June-July water levels and the difference in mean June and mean July water levels. They indicate the amount of error in the estimates for the years considered. Computation of the residuals for Pelican Creek is presented in appendix B. Correlation between the residuals and timing of the runs was not significant ($r = -0.410$, $d.f. = 5$). Hence, the conclusion that timing of the runs was of little importance in controlling Pelican Creek production during the study period appears to be correct.

There is some suggestion that part of the high correlation between water conditions and year-class strength was actually due to escapement, as two years of good production (1953, 1955) had large escapements as well as low water levels. This relation between water levels and escapement is indicated by a correlation of 0.404 (not significant). Figures 3 and 4 show that both June–July water levels and year-class strength were essentially the same in 1948, 1952, and 1954, but that the 1952 escapement (613 females) was less than 10 percent of the 1954 escapement and less than 14 percent of the 1948 escapement. Water levels definitely appeared to be a stronger influence than escapement, at least to the extent that high escapement could not produce a strong year class unless favorable water conditions existed.

Further clarification of the relation of water conditions and escapement to year-class strength is obtained by adjusting year-class abundance to average conditions of water level and water fluctuation. The procedure used for adjusting year-class abundance is as follows. The residuals previously obtained by removing the effect of mean water levels and water-level fluctuations were added to the mean logarithmic value of year-class strength and the antilogs computed (appendix C). The adjusted estimates of year-class strength were then plotted against female escapement (fig. 5). The resulting reproduction curve, drawn in freehand, is a Beverton-Holt type curve (Beverton and Holt, 1957, p. 51) illustrating a population in which year-class abundance is independent of parent escapement over a wide range. Production in Pelican Creek is probably not as independent of spawner escapement as indicated by this curve, but is undoubtedly controlled more by water conditions within the observed range of spawner numbers than by size of the escapement. Under suitable water conditions the reproduction curve may be quite similar to the curve in figure 3.

Chipmunk Creek

There was less evidence of a relation between parent and progeny numbers in the Chipmunk Creek spawning runs ($r = 0.230$, $d.f. = 6$) than in the Pelican

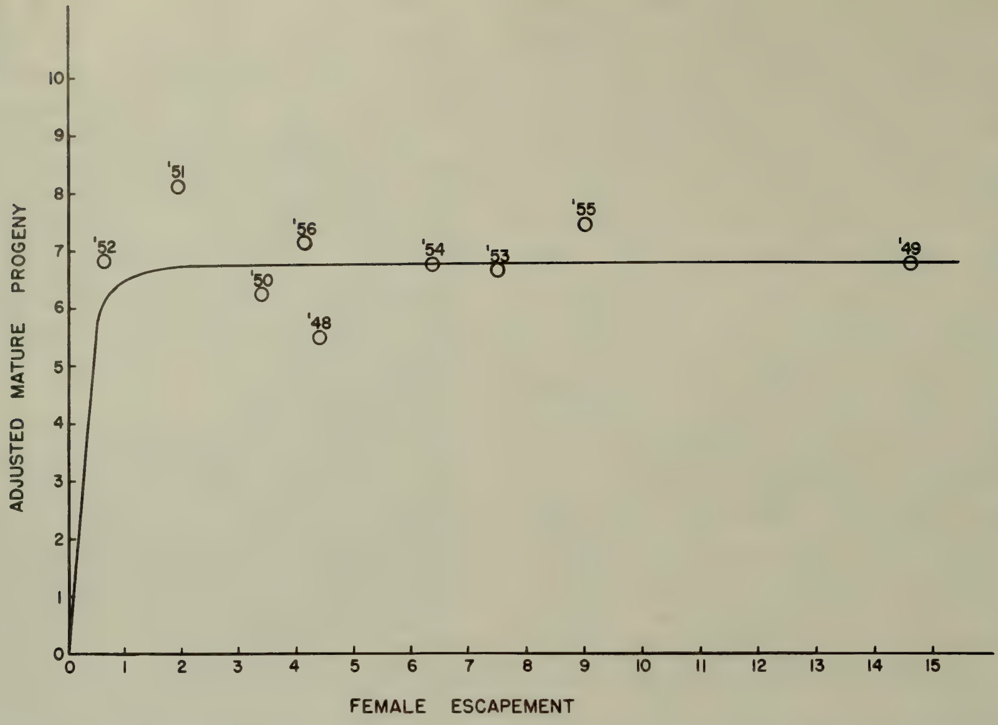

Figure 5.—Pelican Creek reproduction curve with number of progeny adjusted to average water conditions—expressed in thousands of fish.

Creek runs. This lack of significant correlation is apparent from the scatter diagram of Chipmunk Creek escapement and number of fish of age groups III, IV, and V returning from each year class (fig. 6). Recruitment to ensuing runs of progeny from approximately the same number of female spawners in six different years varied from 2,729 to 11,524 fish. Shape of the reproduction curve was not apparent from the data due, in part, to the limited range in number of spawners. The regression line shown was fitted by least squares. The broken diagonal line is based on the average sex ratio of 1.48 females per male for Chipmunk Creek spawners (Ball and Cope, 1961) and indicates the line upon which the parental stock just replaces itself. Escapement to the right of the diagonal line, as indicated by 1950 data, failed to produce equal numbers of progeny. The 1950 escapement containing 2,628 females had a return of only 2,729 progeny, while approximately 4,438 fish of both sexes were needed to maintain the same population density. In 1946 the parental stock just replaced itself. The desirable abundance level of female spawners for Chipmunk Creek appears to be about 2,724 fish, the average number for the eight years examined. This figure represents a spawning run of approximately 4,600 fish based on the average sex ratio of previous runs.

Correlation between year-class strength and mean air temperature during the June-July spawning period was also not significant for Chipmunk Creek ($r=0.100$, $d.f.=6$). There was no relation ($r=0.093$) between year-class strength and mean air temperature during August, the month when stream temperatures were highest in Chipmunk Creek.

Timing of the spawning runs was associated with Chipmunk Creek production, as 45 percent ($r=-0.673$, $d.f.=6$) of the variation in year-class strength was associated with timing although the correlation was not high enough to be significant. From May 28 to July 8, the earlier that 50 percent of the run moved upstream, the greater was the production from Chipmunk Creek. The correlation was not related to the bimodal nature of the Chipmunk Creek runs because the time by which 50 percent of the run had moved upstream did not coincide with the first peak in the number of spawners. Data presented later suggest that the correlation resulted from both variables being influenced by water levels.

Chipmunk Creek averaged 12.7 days later than Pelican Creek for 50 percent of the run to be upstream for the 6 years in which data on timing of the runs were available (table 2). For purposes of analysis the spawning and incubation period was assumed to be 2 weeks later in this stream than in Pelican Creek. There appeared to be no justification from plotted data to transform year-class estimates to logarithmic values, and actual estimates were used. Comparison of Chipmunk Creek year-class strength with mean lake water levels from June 15 to August 15 was not quite significant at the 5-percent level ($r=-0.667$, $d.f.=6$). Failure to obtain a higher correlation suggests either that lake water levels do not accurately represent Chipmunk Creek stream flow or that water level has less control over production in Chipmunk Creek than in Pelican Creek. A correlation, significant at the 5-percent level, between year-class strength and mean July lake levels ($r=-0.755$) indicates that the month of July may be the critical period for water levels in Chipmunk Creek

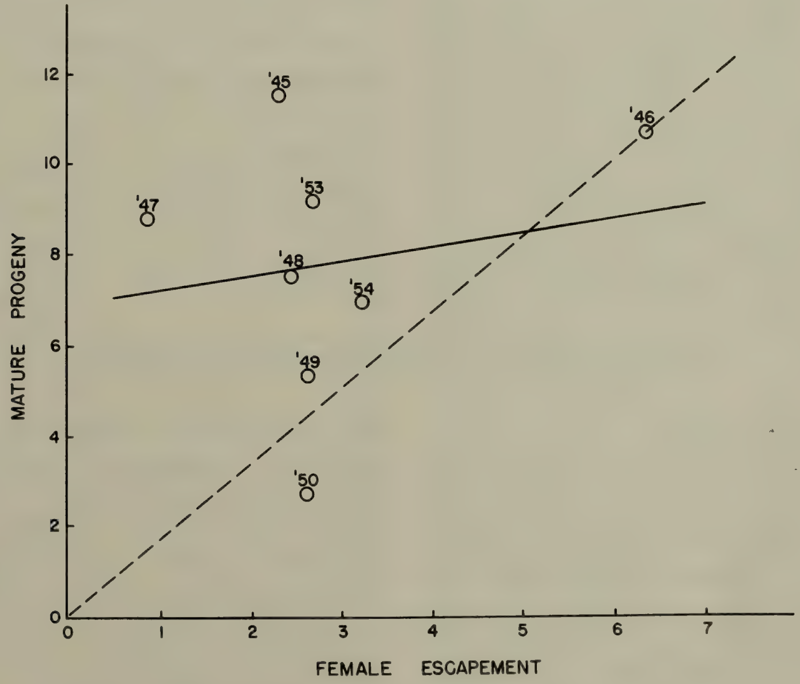

FIGURE 6.—Chipmunk Creek female escapement in relation to the year class it produced—expressed in thousands of fish. Line fitted by least squares. The broken line indicates the level at which replacement of parental stock occurs.

rather than the period from June 15 to August 15.

The amount of fluctuation in water levels within the Chipmunk Creek spawning period was expressed as the difference between mean water levels for the first month of the period (June 15 to July 15) and the second month (July 15 to August 15). The correlation was high but not significant ($r=-0.655$, $d.f.=6$).

Comparison of various combinations of factors with Chipmunk Creek year-class strength did not reveal any significant correlations:

Variables compared	*R*
Water levels, water fluctuations, escapement, timing of runs	0. 834
Water levels, water fluctuations, escapement	. 817
Water levels, water fluctuations, timing of runs	. 799
Water levels, water fluctuations	. 794

Mean water levels from June 15 to August 15 were used in this analysis because the 2-month period covered the time of major incubation in all years. Escapement and timing of the runs contributed little to the multiple correlation, even though timing was almost significantly correlated with year-class strength when compared alone ($r=-0.673$). Mean water levels from June 15 to August 15 and the difference in mean water levels between the first and second month of this period contributed most to the multiple correlation ($R=0.794$), but could still account for less than half of the variation in year-class strength ($R^2_A=0.483$).

A significant correlation between water levels and timing of the runs ($r=0.794$, $d.f.=6$) explains why more of the variation in year-class strength was not accounted for when both variables were considered together with year-class strength. Although water levels from June 15 to August 15 did not coincide exactly with time of the spawning runs, the high correlation suggests a causal relation between water levels and timing. Workers operating the fish traps in both Pelican and Chipmunk Creeks always expect an increase in the runs following a peak in stream water levels. Cope (1956) reported that diurnal fluctuations in stream temperature and water flow triggered upstream migration of spawners in Arnica Creek, another tributary to Yellowstone Lake. Stream discharge frequently influences the time of migration of spawning salmonids (Shapovalov and Taft, 1954), and artificial freshets have even been used to trigger spawning runs of Atlantic salmon (Hayes, 1953). Water levels in Chipmunk Creek influenced timing of the spawning runs as well as year-class strength.

PREDICTION OF FUTURE PRODUCTION

Recruitment to the fishery

Knowledge of factors associated with year-class strength is important for estimating future recruitment to the fishery. Stream flow during spawning appears to be the major factor controlling production in Pelican Creek and Chipmunk Creek although the actual means by which it accomplishes this control is not known. The method used for determining the effect on year-class strength of stream flow and other environmental factors introduced an unknown amount of bias which can be detected only by reference to future data. Hence, forecasts of future recruitment based on the observed correlations must be viewed with caution. Chipmunk Creek correlations were inadequate for prediction, but correlations between lake water levels and Pelican Creek production were sufficiently high that numerical estimates of year-class strength can be calculated for this stream. The values obtained are estimates of the number of initial spawners to expect from a year class. As the fishery centers on fish of essentially the same age groups as found in the spawning runs, the estimates also provide an index of relative year-class strength in that portion of the lake fishery supported by fish from the stream. The estimates should be reliable in the future if our assumption that escapement is of secondary importance is correct and overfishing does not reduce spawning runs below the levels observed during this study.

Pelican Creek estimates are valuable because year-class strengths of Pelican Creek and the Fishing Bridge area fishery are similar, and the Fishing Bridge area accounts for over 60 percent of the total annual catch from Yellowstone Lake. It was necessary to use another method for estimating year-class strength to illustrate this similarity. The method used for spawning runs could not be applied to the fishery because the catch fluctuated widely from 1950 to 1959 and the size of the catchable population was not known. The el-Zarka (1959) method based on the relative contributions of two consecutive year classes was used (appendix D). Relative year-class strength estimates from Pelican Creek spawning runs and Fishing Bridge catch are significantly correlated ($r=0.825$, $d.f.=6$), and either can be used as an index of year-class strength for both. Samples from both areas indicated that 1949 and 1953 produced good year classes and that 1947, 1951, and 1954 produced poor year classes. Factors that control year-class strength in Pelican Creek obviously influence other spawning streams contributing to the Fishing Bridge area fishery. Hence, estimates of Pelican Creek year-class strength should also indicate the relative amount of recruitment to the fishery that can be expected from each year class.

The prediction equation for Pelican Creek utilizes the mean lake water levels during spawning and incubation and differences between mean water levels during this period. The equation is expressed as follows:

$$\hat{Y}=5.90487-0.42450X_1-0.18625X_2$$

where $\hat{Y}$ is $\log_{10}$ year-class strength expressed as the number of fish of age groups III and IV recruited to the spawning runs from year class, X_1 is the mean June–July lake level in feet, X_2 is the difference in feet between mean June and mean July lake levels.

Comparison of estimated year-class strength (geometric mean estimates) with empirical values indicates that the trend is very similar in most years (fig. 7). Estimated strength of the 1948 year class was 23 percent higher than the actual value. Error of the estimates in other years was less, but sufficient to indicate that estimates of future year-class strength must be accepted with due caution.

Recruitment of cutthroat to the Yellowstone Lake fishery commences in the third year of life (age group II) and is completed in the fifth year of life. The major contribution of each year class occurs during the fifth year of life at age IV (Bulkley, 1961). Thus, the 1961 fishery will draw mainly from the 1957 year class, and the 1962 fishery from the 1958 year class. Estimates of relative year-class strength from the above formula for 1957 and 1958 in Pelican Creek with 95-percent confidence limits are as follows: 1957, 7,121 (4,971–10,200); 1958, 14,910 (10,150–21,900). Spawner numbers were adequate in these two years, as female escapement was approximately 10,414 fish in 1957 and 4,320 fish in 1958. The confidence limits are so wide partly because they are set about a single value of Y instead of the mean Y value for the given values of X_1 and X_2, but they also indicate the large amount of possible

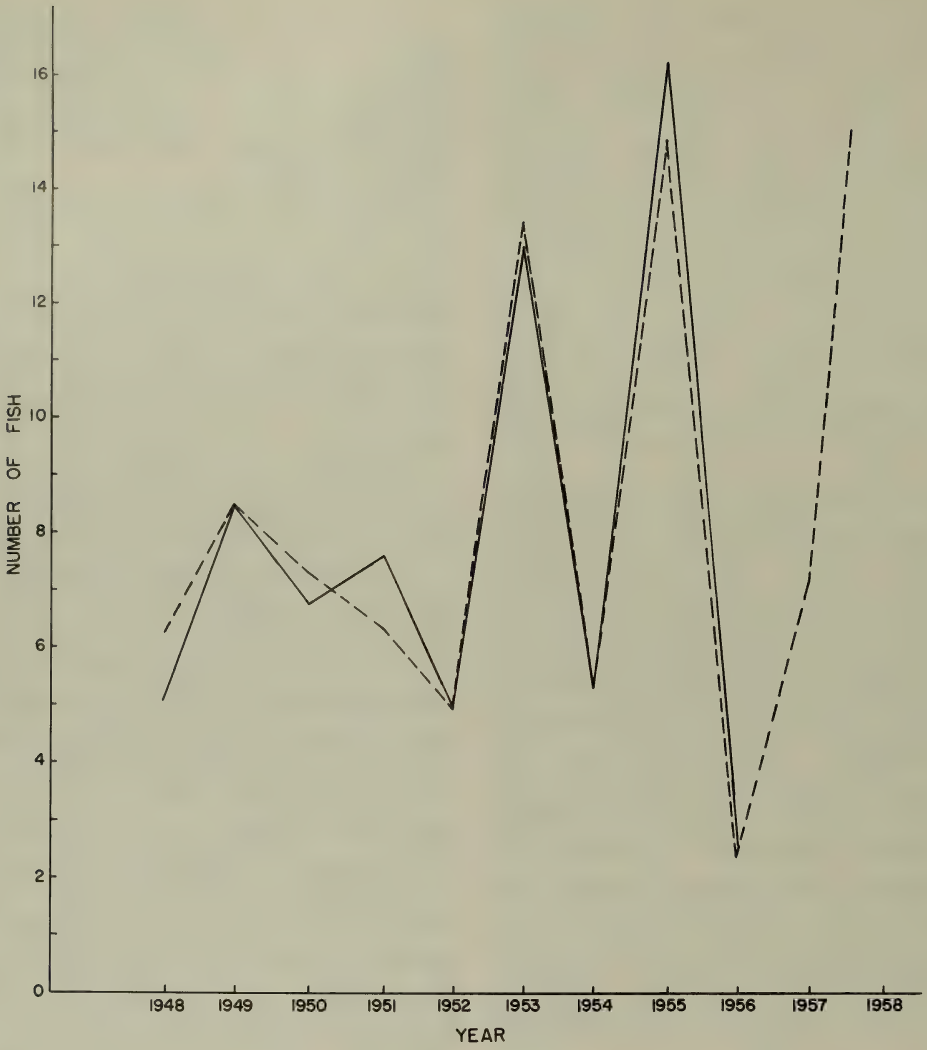

FIGURE 7.—Recruitment to Pelican Creek spawning runs by year class (solid line) and estimated recruitment) recruitment (broken line) from 1948 to 1958—expressed in thousands of fish.

error in the estimates. The 1957 year class was below average strength when compared with data from previous years (fig. 7). This year class was also heavily exploited at age III in 1960[2] so that relatively few fish of age group IV, will be available to the 1961 Fishing Bridge area fishery. The 1958 year class was very strong, and fish of age group III should be relatively abundant in the 1961 fishery. Adequate stocks of fish of age group IV will be available for the fishery in 1962 providing the 1958 year class is not overexploited at age III in 1961.

Numerical estimates of Chipmunk Creek year-class strength are subject to considerable error because only half of the variation in year-class strength can be attributed to known factors. Present data indicate that good production can be expected if water levels are low and relatively stable during the incubation period and if spawner numbers are adequate. Weak year classes will be produced in years when lake water levels are high or fluctuate widely during June and July, even if spawning runs are large. More accurate data are necessary on Chipmunk Creek stream flow and related variables before numerical estimates of year-class strength can be made.

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[2] See footnote 1 on p. 3.

Strengthening of weak year classes

Although the Yellowstone Lake fishery can produce an excellent yield from natural reproduction alone, knowledge of probable year-class abundance at the time the year class is produced provides an opportunity, if desired, to strengthen weak year classes by stocking. As strength of the year class apparently is determined during the spawning and incubation period, supplemental planting of fry after this period would compensate for low survival in years of high water. There is little doubt that fry planting in tributary streams of Yellowstone Lake can be of great benefit.[3] Year classes from 1940 to 1944 in Pelican Creek were entirely the result of fry [4] planting (no escapement allowed), and these year classes were stabler and stronger, on the average, than year classes produced from natural reproduction. It should be noted, however, that the two strongest year classes (1953 and 1955) produced in Pelican Creek from 1942 to 1956 [5] were entirely the result of natural reproduction. In years of favorable water conditions, planting would be unnecessary so long as escapement was adequate.

Data in table 3 provide an index for determining whether planting of fry would be desirable in any given year. Pelican Creek spawners failed to replace themselves with equal numbers of recruits in 1948, 1949, 1954, and 1956. In 1948, 1954, and 1956 mean June–July lake water levels were 4.75 feet or higher, well above the 9-year average of 4.60 feet for this period. In 1949 the mean lake water level was only 4.53 feet, but the escapement of 14,591 females was the largest number ever recorded. Favorable water levels in 1949 should have allowed adequate replacement of spawning stocks. Failure of the run to replace itself is attributed to excessive spawner density in that year. With the high fishing pressure in recent years, we doubt whether excessive escapement will be a future problem. The fact that mean lake levels during spawning were 4.75 feet or higher in three of the four years of insufficient replacement leads us to conclude that very low natural production can be ex-

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[3] See footnote 1 on p. 3.

[4] Some fish were classified as 1-inch fingerlings in the hatchery records.

[5] See footnote 1 on p. 3.

TABLE 3.—*Spawner and recruitment numbers for Pelican and Chipmunk Creeks with related lake water levels*

Year-class	Mean June–July water level	Mean July water level	Pelican Creek			Chipmunk Creek		
			Female escapement	Recruitment, both sexes	Recruitment needed to replace stock[1]	Female escapement	Recruitment, both sexes	Recruitment needed to replace stock[2]
	Feet	*Feet*						
1945	----------	4.24	----------	----------	----------	2,331	11,524	3,906
1946	----------	3.75	----------	----------	----------	6,350	10,665	10,640
1947	----------	4.62	----------	----------	----------	841	8,838	1,409
1948	4.75	4.49	4,418	5,032	7,109	2,449	7,500	4,104
1949	4.53	4.38	14,591	8,377	24,056	2,650	5,286	4,441
1950	4.61	5.34	3,381	6,755	5,440	2,628	2,729	4,404
1951	4.72	----------	1,969	7,569	3,168	----------	----------	----------
1952	4.77	----------	613	4,985	986	----------	----------	----------
1953	3.74	4.27	7,475	12,863	12,027	2,711	9,184	4,543
1954	4.75	5.19	6,438	5,267	10,359	3,237	6,921	5,424
1955	3.95	----------	9,030	16,023	14,529	----------	----------	----------
1956	5.61	----------	4,166	2,503	6,703	----------	----------	----------

[1] Based on average sex ratio of 1.64 females per male.

[2] Based on average sex ratio of 1.48 females per male.

pected in years when this water condition occurs. Supplemental fry planting should significantly increase production in Pelican Creek in these years.

According to available data, Chipmunk Creek spawners failed to replace themselves only in 1950 when the mean July lake level (5.34 feet) was the highest recorded. The next highest mean July level was 5.19 feet in 1954, when the spawning run more than replaced itself. The critical July water level for Chipmunk Creek production must lie between 5.19 and 5.34 feet. Supplemental stocking of fry would appear to be beneficial in this stream whenever mean July lake level exceeds 5.19 feet.

Reliable forecasts of lake water levels are essential in a management program based on water conditions where fish planting is desirable in some years and not in others; preparations must be made in advance for the taking of spawn and for hatchery operations. Since 1937 the U.S. Soil Conservation Service, in cooperation with the U.S. National Park Service, has conducted a snow survey at several points in the Yellowstone Lake drainage. By relating snow survey measurements taken over a period of years to spring-summer runoff during the same period, relations have been developed which make it possible to forecast seasonal runoff several months in advance of occurrence. Snow survey data from 1937 to 1960 were utilized by the Soil Conservation Service at Bozeman, Mont., to compute formulas for accurately predicting Yellowstone Lake water levels in June and July as early as March 1 of the same year. Accuracy of the forecasts is influenced by the amount of precipitation during the spring and early summer and by air temperatures during the melt season. The forecasts are sufficiently accurate to determine whether water conditions will be suitable for cutthroat reproduction and whether fry stocking would be advisable.

Forecasts of Yellowstone Lake water level during 1961 based on snow conditions as of March 1, 1961, are as follows: [6] The expected mean June-July lake water level will be approximately 3.94 feet, well below the level where supplemental planting is recommended (4.75 feet). Expected mean June–July lake water level based on snow conditions as of April 1, 1961, is 3.87 feet, slightly lower than the March 1 estimate but essentially the same. Conditions for trout reproduction in Pelican Creek should be excellent in 1961. The mean July lake water level is predicted to be 4.07 feet above datum based on March 1 snow conditions and 4.05 feet according to snow conditions on April 1.[7] Favorable water conditions are also indicated for Chipmunk Creek production if runoff is gradual. These water forecasts suggest that fry stocking is unnecessary in 1961 and that a strong year class will be produced, but inadequate spawner escapement may seriously reduce production. A very low escapement is expected in both streams in 1961 owing to the heavy overfishing in 1959 and 1960 in Yellowstone Lake [8] and to the weak 1957 year class in Pelican Creek. If spawner escapement falls below previous levels, the number of spawners may become the major factor limiting production. Data collected in 1961 and ensuing years should cast additional light on the relation of spawner escapement and water levels to year-class strength and allow for the refinement of the prediction formulas.[9]

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[6] Personal correspondence from Mr. Ashton R. Codd, Snow Survey Supervisor, Soil Conservation Service, Bozeman, Mont.

[7] After 1961 forecasts of Yellowstone Lake water conditions based on snow condition on February 1, March 1, and April 1 will appear in "Snow Survey and Water Supply Forecasts for Montana and Northern Wyoming (Upper Missouri and Upper Columbia River Basins)." U.S. Department of Agriculture, Soil Conservation Service, and Montana Agricultural Experiment Station, Bozeman, Mont.

[8] See footnote 1 on p. 3.

[9] The actual number of initial spawners from the 1957 year class which entered the 1960 and 1961 Pelican Creek runs was 8,643, which was 21 percent above the predicted number but well within the established confidence limits.

SUMMARY

Large fluctuations in year-class strength of cutthroat trout occurred from 1948 to 1956 in Pelican Creek and from 1945 to 1954 in Chipmunk Creek. Recruitment to the spawning runs was used as an index of year-class strength.

Recruitment was related to female escapement in Pelican Creek, although the exact shape of the reproduction curve was obscured by environmental influences. Correlation between female escapement and recruitment of the year class was not significant ($r=0.480$). The 1949 run of 14,591 female spawners appeared to be an excessive escapement for Pelican Creek. Maximum production should be obtained from approximately 9,000 females if environmental conditions are suitable. The date by which 50 percent of the Pelican Creek run moved upstream was not a critical factor in production ($r=0.115$). Mean air temperature during the spawning period ($r=0.325$) and during the month of maximum stream temperature ($r=0.049$) were also not major factors.

Mean lake level during June and July which was used as an index of stream flow was strongly correlated with Pelican Creek year-class strength ($r=-0.955$). Variation in year-class strength associated with mean water levels and the difference in water levels during spawning amounted to 94.3 percent ($R=0.978$). The analyses indicate that adequate escapement cannot produce an abundant year class unless water levels are favorable.

Chipmunk Creek year-class strength appeared to be completely independent of escapement ($r=0.230$) within the range of 841 to 6,350 female spawners. The average escapement of 2,724 females appeared to be adequate. The time by which 50 percent of the run moved upstream was correlated with Chipmunk Creek year-class strength ($r=-0.673$), but this relation was not causal because water levels influenced both timing of the runs and year-class strength. Air temperatures during the spawning period ($r=0.100$) and during August when stream temperature was highest ($r=0.093$) were not critical factors. Multiple correlation between year-class strength, mean water level, and fluctuations in water levels during the incubation period showed that less than 50 percent of the variation in year-class strength ($R=0.794$) was associated with these two factors.

Recruitment to the Pelican Creek spawning runs from the 1957 and 1958 year classes was estimated to be 7,121 and 14,910 fish respectively from the formula $\hat{Y}=5.90487-0.42450X_1-018625X_2$. Year-class strength in Pelican Creek and the Fishing Bridge area fishery are similar. The number of age IV fish in the fishery will be well below average in 1961 and above average in 1962 unless overfishing removes the fish at a younger age. No predictions were made from Chipmunk Creek correlations, but good year classes can be expected if water levels are low and stable during spawning and if escapement is adequate.

Stocking of fry has been successful in past years, which indicates that the large fluctuations in year-class strength can be leveled off by fry-planting in years of unsuitable water conditions. Stocking is recommended in Pelican Creek in years when mean June–July lake level is above 4.75 feet and in Chipmunk Creek when mean July lake level is above 5.19 feet. Acceptable forecasts of lake water levels during incubation, to determine whether stocking is necessary in any given year, can be made as early as March 1 from snow survey data. The forecast for 1961 indicates that water levels should be excellent for trout production if runoff is gradual.

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Appendix A—WATER LEVELS AND AIR TEMPERATURE DATA ON YELLOWSTONE LAKE

TABLE A–1.—*Yellowstone Lake mean water levels and mean air temperatures, 1945 to 1956*

Factor and period	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956
Water level (feet):												
June 1–15	3. 65	3. 54	3. 70	4. 61	4. 31	3. 10	4. 02	4. 67	2. 24	3. 96	2. 84	5. 61
16–30	5. 09	4. 16	4. 57	5. 39	5. 05	4. 67	4. 86	5. 88	4. 18	4. 65	4. 22	6. 38
July 1–15	4. 11	4. 01	4. 73	4. 94	4. 77	5. 59	5. 09	4. 54	4. 52	5. 45	4. 34	5. 70
16–31	4. 36	3. 49	4. 51	4. 03	3. 98	5. 08	4. 89	4. 00	4. 01	4. 92	3. 88	4. 73
August 1–15	3. 74	2. 83	3. 85	3. 22	3. 19	4. 05	4. 53	3. 35	3. 25	3. 85	3. 29	3. 81
Air temperature (°F.):												
June 1–30	43. 1	48. 8	43. 6	50. 2	46. 8	44. 8	41. 7	48. 7	46. 2	43. 7	45. 3	54. 0
July 1–31	51. 0	56. 2	55. 8	58. 0	54. 1	50. 8	55. 4	52. 0	57. 1	61. 8	56. 3	55. 6
August 1–31	53. 0	53. 1	52. 6	59. 0	54. 4	51. 6	52. 9	52. 8	54. 1	51. 3	56. 4	52. 7

Appendix B—COMPUTATION OF RESIDUALS

TABLE B–1.—*Calculations used to compute residuals for Pelican Creek year-class strength with effect of annual and monthly water-level fluctuations removed*

Year	Number of fish in age groups III and IV returning	Log $_{10}$ Y	Mean June-July water levels X_1	Difference between mean June and mean July water levels X_2	Calculated [1] $\hat{Y}$	Residuals $Y-\hat{Y}$
1948	5, 032	3. 70174	4. 75	0. 51	3. 79352	−0. 09178
1949	8, 377	3. 92309	4. 53	0. 30	3. 92602	−0. 00293
1950	6. 755	3. 82963	4. 61	1. 45	3. 86413	−0. 03450
1951	7. 569	3. 87904	4. 72	0. 55	3. 79880	0. 08024
1952	4. 985	3. 69767	4. 77	1. 01	3. 69191	0. 00576
1953	12, 863	4. 10934	3. 74	1. 06	4. 11984	−0. 01050
1954	5, 267	3. 72156	4. 75	0. 88	3. 72461	−0. 00305
1955	16, 023	4. 20474	3. 95	0. 33	4. 16665	0. 03809
1956	2, 503	3. 39846	5. 61	0. 78	3. 37816	0. 02030

[1] Calculated from the formula $\hat{Y}=5.90487-0.42450X_1 - -0.18625X_2$

Appendix C—ADJUSTED ESTIMATES OF PELICAN CREEK YEAR-CLASS STRENGTH

TABLE C–1.—*Calculation of year-class strength for average June-July lake water levels and average change in mean water levels from June to July*

1 Year	2 Log residuals	3 Female escapement	4 Log adjusted numbers [1]	5 Adjusted year-class strength [2]
1948	−0. 09178	4, 418	3. 73769	5, 457
1949	−0. 00293	14, 591	3. 82654	6, 707
1950	−0. 03450	3, 381	3. 79497	6, 237
1951	0. 08024	1, 969	3. 90971	8, 123
1952	0. 00576	613	3. 83523	6, 843
1953	−0. 01050	7, 475	3. 81897	6, 591
1954	−0. 00305	6, 438	3. 82642	6, 705
1955	0. 03809	9, 030	3. 86756	7, 371
1956	0. 02030	4, 166	3. 84977	7, 076

[1] Mean $\log_{10}$ year-class strength=3.82947. Column 4=3. 82947 plus column 2.

[2] Antilog of column 4.

Appendix D—RELATIVE YEAR-CLASS STRENGTH OF PELICAN CREEK AND FISHING BRIDGE CATCH

The method of el-Zarka (1959) was used to obtain estimates of relative year-class strength to compare Pelican Creek fish with the catch at Fishing Bridge dock. This method is based on the relative contributions to the catch or spawning runs of two consecutive year classes. Only age groups III, IV, and V were used for the comparison, because age group II is not present in the spawning runs, and the older age groups are not always well represented in the catch. Data from table D–1 will be used to illustrate the method. The 1947 year class at Pelican Creek in its fourth year of life comprised 4.6 percent of the 1950 spawning run in that stream, 55.4 percent of the 1951 run in its fifth year of life, and 22.0 percent of the 1952 run in its sixth year of life. Sum of the three percentages is 82.0 percent. The 1948 year class in comparison contributed at the same ages; 2.5 percent of the 1951 run, 60.3 percent of the 1952 run, and 29.6 percent of the 1953 run. Total for the 1948 year class was 92.4 percent. The percentage difference in strength of the two year classes was then determined in the following manner:

$$\frac{2\ (82.0-92.4)\times 100}{82.0+92.4}=11.9$$

Hence, the 1948 year class was approximately 11.9 percent larger than the 1947 year class. Comparisons and calculations used to rank all year classes are summarized in table D–2. The percentage differences between years were first adjusted to give the relative position of each year class in the series. This adjustment was made by arbitrarily assigning year class 1947 a value of 0.0 and adding percentage differences of each succeeding comparison to it. The resulting series of figures was then adjusted to a mean of 0.0 for all year classes to obtain the final rankings presented in the right-hand column of table D–2.

Table D–1.—*Partial age composition and year classes of Yellowstone Lake cutthroat*

[Data from Pelican Creek spawning runs and catch at Fishing Bridge dock, 1950–59]

Year of capture	For year class of—							
	1947	1948	1949	1950	1951	1952	1953	1954
1950:								
Age group	III							
Percentage:								
Pelican Creek	4.6							
Fishing Bridge	13.1							
1951:								
Age group	IV	III						
Percentage:								
Pelican Creek	55.4	2.5						
Fishing Bridge	34.0	13.5						
1952:								
Age group	V	IV	III					
Percentage:								
Pelican Creek	22.0	60.3	14.3					
Fishing Bridge	23.4	39.2	30.9					
1953:								
Age group		V	IV	III				
Percentage:								
Pelican Creek		29.6	58.3	8.6				
Fishing Bridge		35.3	30.8	21.3				
1954:								
Age group			V	IV	III			
Percentage:								
Pelican Creek			37.0	55.0	3.4			
Fishing Bridge			39.3	30.1	18.8			
1955:								
Age group				V	IV	III		
Percentage:								
Pelican Creek				32.4	58.2	6.5		
Fishing Bridge				41.3	26.9	20.1		
1956:								
Age group					V	IV	III	
Percentage:								
Pelican Creek					32.4	59.7	5.5	
Fishing Bridge					33.5	43.0	18.4	
1957:								
Age group						V	IV	III
Percentage:								
Pelican Creek						26.0	71.3	1.6
Fishing Bridge						30.8	53.5	12.3
1958:								
Age group							V	IV
Percentage:								
Pelican Creek							27.6	68.7
Fishing Bridge							38.1	46.3
1959:								
Age group								V
Percentage:								
Pelican Creek								23.3
Fishing Bridge								17.4

Table D–2.—*Data employed in determination of relative strength of year classes 1947 to 1954 of Yellowstone Lake cutthroat in Pelican Creek spawning runs and the catch at Fishing Bridge dock*

[Age groups III, IV, and V only]

Year-classes compared	Sum for first year	Sum for second year	Mean	Difference	Percentage difference between years	Relative strength of first year	Final rank of first year
Pelican Creek:							
1947–1948	82.0	92.4	87.2	10.4	11.9	0.0	−14.9
1948–1949	92.4	109.6	101.0	17.2	17.0	11.9	−3.0
1949–1950	109.6	96.0	102.8	−13.6	−13.2	28.9	14.0
1950–1951	96.0	94.0	95.0	−2.0	−2.1	15.7	0.8
1951–1952	94.0	92.2	93.1	−1.8	−1.9	13.6	−1.3
1952–1953	92.2	104.4	98.3	12.2	12.4	11.7	−3.2
1953–1954	104.4	93.6	99.0	−10.8	−10.9	24.1	9.2
1954						13.2	−1.7
Fishing Bridge:							
1947–1948	70.5	88.0	79.3	17.5	22.1	0.0	−22.2
1948–1949	88.0	101.0	94.5	13.0	13.7	22.1	−0.1
1949–1950	101.0	92.7	96.8	−8.3	−8.6	35.8	13.7
1950–1951	92.7	79.2	86.0	−13.5	−15.7	27.2	5.1
1951–1952	79.2	93.9	86.6	14.7	17.0	11.5	−10.7
1952–1953	93.9	110.0	102.0	16.1	15.8	28.5	6.4
1953–1954	110.0	76.0	93.0	−34.0	−36.5	44.3	22.2
1954						7.8	−14.4

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LIMNOLOGICAL ORGANIC ANALYSES BY QUANTITATIVE DICHROMATE OXIDATION

John A. Maciolek
Fishery Research Biologist

RESEARCH REPORT 60

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ABSTRACT

A limnological oxidation technique, based on procedures employed in soil chemistry, was developed and tested by use of purified organic compounds and various natural substrates. A sample is oxidized by dichromate in a strongly acid medium (2 H_2SO_4 to 1 H_2O, by volume) maintained at 100° C. The amount of dichromate reacting (determined titrimetrically as the difference between the initial and final quantities) is reckoned as Oxygen Consumed, or weight of oxygen required by the sample. The procedure is adapted to samples of different sizes by adjusting the volume or normality of the dichromate. Under optimal conditions, resistant compounds are oxidized with an efficiency of 95 percent or more. The oxidation also serves as the principal step for sensitive determinations of organic carbon (as CO_2) and nitrogen (as NH_3). Both elements are estimated by titration after diffusion separation—the carbon absorbed in hydroxide concurrent with oxidation and the nitrogen diffused subsequently from the reacted mixture into a boric acid solution. These analyses require no special apparatus or complicated procedures. They are sensitive to as little as 0.01 milligram of organic matter, precise to within ± 1 percent in the semimicro range, and adaptable to a variety of sample materials and to multiple simultaneous determinations.

LIMNOLOGICAL ORGANIC ANALYSES BY QUANTITATIVE DICHROMATE OXIDATION

By John A. Maciolek, *Fishery Research Biologist*
Bureau of Sport Fisheries and Wildlife

Part I. INTRODUCTION

Estimation of the quantity of organic matter in aquatic samples is difficult because of its different physical states and its low concentration relative to mineral matter and water. Probably the most common primary measurement of total organic matter is a mass estimate made by determining the loss in weight upon ignition of a sample that has been evaporated to dryness. Ignition loss, being an indirect measurement, carries an error proportional to the amount of inorganic matter present, and this error is usually too large for meaningful interpretation of analytical results.

In some circumstances, the determination of energy rather than mass is a more important primary measurement of sample organic matter (cf. MacFadyen, 1949). The direct estimate of organic energy, as done by bomb calorimetry, is difficult to adapt to limnological samples. This technique also has the disadvantages of being analytically exacting and of not allowing rapid or multiple simultaneous determinations.

Various other measurements provide information which more fully describes the organic nature of a sample. Aquatic organic matter has been characterized in this manner by specific molecular or elemental constituents, and by its proximate composition. These determinations involve techniques such as chromatography, spectrophotometry, combustion analysis, and solvent extraction. Limnological adaptions of standard analytical methods may be criticized as having insufficient sensitivity or accuracy, or as being too complicated for the skills and facilities of the average researcher. Dissolved organic matter, especially, has been inadequately studied for lack of a simple, direct method of analysis. The key problem in investigating aquatic organic matter, then, is methodological.

Need for a better method of measuring total organic matter arose particularly in connection with continuing investigations of strongly oligotrophic lakes in the Sierra Nevada of California. Net plankton was not detected in some of these lakes (Reimers et al., 1955) and in no case did ignition loss exceed 6 milligrams per liter. At the lower extreme, the concentration of total dissolved solids in a small cirque lake (Reimers, 1958) was about 8 milligrams per liter, and the ignition loss was only 0.2 milligrams per liter. The situation required an analytical method that was simple, sensitive to low levels of organic matter, and preferably adaptable to multiple simultaneous determinations. The original objective in developing such a method was to permit within-lake and between-lakes comparisons of total organic matter in terms of mass or energy. The principle of quantitative oxidation, or decomposition of a sample with a measured amount of oxygen, appeared to be a suitable basis upon which to develop the desired method.

Quantitative oxidation analysis is based on the reduced state of organic matter. Consider a "typical" molecule as metabolically constructed from CO_2, H_2O, and NH_3 by removal of some of the oxygen atoms and condensation of the remainder into a unit having a framework of carbon with all of the atoms covalently bonded. The ratio of these atoms with respect to their valences must establish electronic neutrality within the molecule. The formation process is one of reduction and requires energy in proportion to the degree of reduction. This process is reversed in chemical oxidation to yield the original inorganic constituents. The amount of oxygen required is proportional to the energy content and is also an indirect estimate of the quantity of organic matter oxidized.

Quantitative oxidations are most conveniently accomplished with combined oxygen, that is, in relatively stable reagents which can be reduced by organic matter under certain reaction conditions. A known excess of oxidant is provided, and the remainder is determined after the reaction has been completed. The amount of reacting oxygen is the equivalent difference between the two values. These reactions, whether quantitative or not, are commonly referred to as "wet oxidations."

Appraisal of existing methods indicated that the oxidative reaction could also serve as an initial step in procedures for analyzing component elements. The problem was therefore expanded to include the development of simple accessory analyses for carbon and nitrogen as well as the necessary interpretation of all empirical results in terms of natural organic matter. This analytical scheme is propounded here as an approach to the general characterization of organic matter in all types of limnological samples.

Part II. OXIDANTS AND OXIDATIVE PROCEDURES

An extensive survey was made of literature concerning the analysis of organic matter by oxidative methods in order to determine the most suitable reagents and procedures for quantitative limnological oxidations. Primary emphasis was placed on hydrochemical techniques and on reports concerning quantitative oxidation with dichromate, and the survey was intensive in this respect.

There are many reagents which can serve as organic oxidants, but these cannot be compared solely on a theoretical basis since their characteristics will vary under different reaction conditions; comparisons must be made with respect to the procedures in which they are used. In terms of the objectives of this study, the desirable characteristics of a procedure for quantitative oxidation are—

1. Efficiency and accuracy; organic matter should be completely oxidized as rapidly as possible with the least interference from inorganic constituents.

2. Sensitivity and precision; differences between samples should be distinguishable at a microanalytical level with a minimum of replication.

3. Simplicity and versatility; the procedure should require a minimum of essential equipment and operations as well as numbers and amounts of reagents, and should apply equally well to dissolved or particulate matter over a wide range of sample sizes, regardless of the amount of inorganic matter present.

PERMANGANATE AND OTHER OXIDANTS

Potassium permanganate has been employed as a quantitative oxidant in hydrochemistry for more than a century. A comprehensive review of its history, methodology, and effectiveness in organic oxidation was presented by Meissner (1958). Meissner attributed the introduction of permanganate methods of water analyses to Forschammer in 1849, when Forschammer was attempting to overcome the limitations of ignition loss gravimetry. According to Mason (1890), Forschammer's original technique merely required the addition of standard permanganate solution to a water sample until its color persisted. Subsequent improvements consisted in adding acid or hydroxide to the sample, and heating the mixture to increase the reaction rate (Kubel technique). Meissner (1958) stated that none of the many method variations proposed significantly improves upon the 70-year-old Kubel technique. More recent literature dealing with limnological methods and applications of permanganate oxidation includes Åberg and Rodhe (1942), Berg and Petersen (1956), Braun (1952), Dobrzhanskaya (1956), Elster (1959), Gillbricht (1957), Juday and Birge (1932), Matsue (1950), Maucha (1932), Ohle (1940), and Wereščagin et al. (1932). Generally, oceanographic investigators have found that permanganate methods are inapplicable to sea-water analyses (Lopez-Benito, 1955; Duursma, 1960).

Permanganate has several disadvantages as an analytical reagent (Kolthoff and Sandell, 1952), the worst being its tendency toward autodecomposition. Because of this instability, it cannot be employed in the rigorous reaction conditions necessary for complete oxidation of organic matter. Despite the many methods proposed, permanganate usually oxidizes less than half of the total organic matter present. Juday and Birge (1932) concluded that the permanganate method they used was about 40 percent effective in oxidizing organic carbon. Permanganate and dichromate oxidations were used by Votintsev (1953, 1955) in analyzing Lake Baikal samples, from surface foam to a depth of 1,200 meters. His data show that the oxidation values obtained with permanganate were only 10 to 54 percent of those yielded by dichromate.

The oxidative effectiveness of permanganate in both acid and alkaline reaction was compared with that of acid dichromate using the procedure described in part IV. Samples of filtered hatchery water, analyzed by the three methods, gave the results in table 1. Since the dichromate oxidation was approximately 90 percent complete, permanganate oxidized only about 30 percent of the organic matter present.

Low and erratic results of permanganate oxidations with natural organic matter can be better understood from the data of Meissner (1958), which concern the permanganate oxidizability of more than 200 organic compounds. Not only are the recoveries generally low, but there is no consistency of results within the various groups of compounds (amino acids, carbohydrates, fatty acids).

TABLE 1.—*Results from permanganate and dichromate oxidations of dissolved organic matter in trout-hatchery water at Cortland, N.Y.*

Sample analyzed	Oxygen consumed, milligrams per liter [1]		
	Acid permanganate	Alkaline permanganate	Acid dichromate
Influent (supply stream)	0.63	0.10	1.86
Effluent (from raceways)	1.51	1.06	4.53

[1] All values are unadjusted means of duplicate determinations. The acid permanganate method was that used by Juday and Birge (1932), the alkaline permanganate method is from Maucha (1932), and the dichromate oxidation is described in this report under "Micro method."

Certain other oxidants and methods of oxidation are worthy of note in that they pertain either to water analysis or to the treatment given organic matter in this study. Medalia (1951) has suggested an interesting and sensitive partial oxidation for detecting traces of organic matter in water. It utilizes ferrous iron and hydrogen peroxide as reagents, and these react with certain organic linkages in a chain reaction which oxidizes both the iron and the organic linkage. More recently, Slater (1954) described a technique for quantitatively oxidizing organic matter in natural waters with ammonium nitratocerate and perchloric acid. Ceric sulfate, periodate, and other oxidants have been used effectively in quantitative organic oxidations (Buydens and Ledent, 1957; Skopintsev, 1949c, 1950; Urbanowiczówna, 1947).

Dry combustion methods may also serve as quantitative oxidations, but the procedures are generally more difficult. Krogh (1930) employed a cupric-oxide combustion on dried organic residues, with gasometry to measure the end products and the oxygen needed to reoxidize the copper. The proximate composition and energy content of the sample were calculated from these values. Krogh has applied this method limnologically (Krogh and Berg, 1931; Krogh and Lange, 1932). Ultimate analysis by a standard dry combustion-absorption train enabled Spoehr and Milner (1949) to calculate indirectly the oxygen required for combustion of algal samples. This oxygen was expressed as the "R-value" and was used to determine the proximate composition of the samples.

DICHROMATE

Chromic acid and its salts have been used effectively in oxidizing organic matter by procedures developed in a number of investigational fields. Potassium dichromate, in particular, appears to be the most suitable oxidant for the present problem and offers the following advantages:

It is a primary standard.

It is very effective in oxidizing most organic compounds under suitable reaction conditions.

It is extremely stable in neutral solution, and also in acid solution if protected from contamination.

It does not decompose spontaneously to any appreciable extent during a reaction, even at high temperatures and acid concentration.

It can be measured colorimetrically or titrated by several common reductants to a very sharp endpoint.

It can be used in procedures which require no unusual precautions.

It contains (or requires for reaction) no elements which are known to interfere with the accessory analyses considered later.

One of the earliest applications of dichromate in organic oxidations was reported by Rogers and Rogers (1846, 1848) who found it superior to "peroxide of manganese" in preparative reactions for formic and acetic acids. Subsequently, many dichromate techniques were developed for biochemical analyses, such as the quantitative oxidations described by Christensen et al. (1937), Ivlev (1934), Johnson (1949), and Kuhn and L'Orsa (1931).

Various hydrochemical analyses employing dichromate have appeared in recent years. Direct carbon determinations by nonquantitative oxidation was described by Forsblad (1955), Kay (1954), Krogh and Keys (1934), Krylova (1953), Mohlman and Edwards (1931), and Skopintsev (1949a). Quantitative oxidations have been developed for the sanitation-pollution field (A.P.H.A., 1955; Moore et al., 1949; Moore and Walker, 1956). In limnology, quantitative dichromate analyses have been developed primarily in Russia. Vinberg et al. (1934) designed a simple technique and employed it on a unique study of the benthos of Lake Beloje. Vinberg's technique has been modified and adapted for the analysis of dissolved organic matter, one of the more recently described methods being that of Nikolaeva (1953). The studies of Votintsev (1953, 1955) on Lake Baikal provide an example of the application of dichromate oxidation to the measurement of dissolved organic matter. Another simple dichromate technique for estimating organic carbon in water samples was given by Šesták (1958). Procedures for marine analyses were described by El Wakeel and Riley (1957) and Strickland and Parsons (1960).

Analytical problems similar to those of limnology are encountered in the field of soil chemistry where the history of oxidative analysis for total organic matter and organic carbon has been traced by Clark and Ogg (1942). Dichromate was first employed as a combustion reagent in the direct determination of organic carbon (Ames and Gaither, 1914). Modifications of this procedure are still being proposed (Shaw, 1959; Allison, 1960). The use of a quantitative oxidation to determine soil organic content is generally attributed to Schollenberger. His "rapid approximate" method (Schollenberger, 1927) has been variously modified (Allison, 1935; Graham, 1948; Schollenberger, 1945; Tiurin, 1931; Walkley and Black, 1934). Perhaps the simplest and most widely used technique of this type is the one described by Peech et al. (1947).

A GENERAL PROCEDURE FOR DICHROMATE OXIDATION

Methods described in the literature were evaluated to select the best procedure upon which to develop the desired analytical techniques. Oxidation of a sample is commonly accomplished with a large excess of dichromate and a high concentration of sulfuric acid. Heat is employed to hasten the reaction, and the excess dichromate is determined titrimetrically after diluting the reactants. Procedures differ in several minor respects that make them more or less acceptable. Some objectionable features noted were inherently poor precision or accuracy, the need for special apparatus, and the lack of sensitivity or adaptability to samples of various types and sizes. "Rapid" methods used in soil analysis seemed most desirable because of their simplicity, versatility, and potential usefulness in limnology.

The following is a general outline of the oxidation procedure which is of concern in the remaining text. It is based on the Walkley method (Peech et al., 1947), initially modified by extending the reaction time in a boiling water bath to enable more effective oxidation of resistant compounds.

1. A sample (moist, dry, or dispersed in a small quantity of water) is placed in a suitable heatproof flask.

2. A known amount of potassium dichromate is added to the sample in excess of that required for complete oxidation.

3. Concentrated sulfuric acid is added in an amount equal to twice the volume of water present, and the flask is swirled.

4. The flask is lightly covered and placed in a boiling water bath until the reaction is considered complete.

5. The reaction mixture is diluted and cooled, and its excess oxidant is titrated (ferrous solution). Oxygen consumed is derived from the difference between sample and reagent blank titers.

Part III. REACTION CHARACTERISTICS OF DICHROMATE AND ORGANIC MATTER

All organic compounds are susceptible to oxidation by dichromate under certain conditions. These conditions impose restrictions on the design of a suitable quantitative procedure. Because the reaction is neither simple nor instantaneous, consideration must be given to the properties of the reactants and to the factors which affect their reactive efficiency. The analysis must be expressed quantitatively according to the relation between organic matter and oxygen, reacting as dichromate. Two expressions (Oxygen Equivalent and Oxygen Consumed) are proposed to satisfy both theoretical and empirical oxygen-organic matter relations.

PROPERTIES OF DICHROMATE

Dichromate ions ($Cr_2O_7^{-2}$) form orange-colored solutions which reduce to green or bluish-violet chromic (Cr^{+3}) derivatives. They are strong oxidizing agents in the presence of free acids. The proton requirement in this reaction is given by the ionic equation

$Cr_2O_7^{-2} + 14H^+ + 6e \rightarrow 2Cr^{+3} + 7H_2O$, which is generalized because ions of intermediate valence occur in the reduction of chromate to chromic chromium. Reagent-grade potassium dichromate (molecular weight = 294.21) is a primary standard; its equivalent weight, indicated in the reaction above, is 294.21/6 or 49.04. The standard oxidation potential, E_o (at 25° C. with reference to *N* hydrogen electrode, pH = 0), of this reaction is about − 1.35 volts. The actual oxidation potential of any dichromate system varies with temperature, pH, and dichromate-chromate ion concentrations according to the relations

$$E = E_o + \frac{0.0001983\ T}{6} \log \frac{(H^+)^{14}\ (Cr_2O_7^{-2})}{(Cr^{+3})^2}$$

where E = observed potential, E_o = standard potential, and T = absolute temperature.

Dichromate is essentially an acid salt. It reacts with hydroxyl ions to form yellow chromates (CrO_4^{-2}) and water. Chromic ions (from reduced dichromate) also react with hydroxyl ions to produce green chromites ($Cr(OH)_4^{-1}$). These alkaline reactions do not involve electron changes and therefore are not oxidations. They will be referred to later in connection with neutralizing the reaction mixture.

The stoichiometric relations involved in the titration of dichromate with ferrous sulfate are expressed by the equation $6FeSO_4 + K_2Cr_2O_7 + 7H_2SO_4 \rightarrow K_2SO_4 + 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + 7H_2O$. The relation between oxygen and a reductant in an oxidative reaction is indicated also. Six equivalents of oxygen, as dichromate, are required for the complete oxidation of 6 moles (or 6 equivalents) of ferrous sulfate. Since the equivalent weight of oxygen is 8, and that of ferrous sulfate is 151.92, 8/151.92 or 0.0527 represents the weight of oxygen required per unit weight of ferrous sulfate. This ratio (eq. wt. oxygen/eq. wt. reductant), with equivalents expressed in the same units, is referred to hereafter as the Oxygen Equivalent (O.E.) of a reductant. Oxidizable compounds or mixtures of such compounds are distinguishable by their characteristic O.E.'s. Oxygen Equivalents can be determined experimentally as well as mathematically. The weight of oxygen used in a reaction, divided by the weight of the substrate reduced (pure compound or mixture), is the fundamental relation of analysis by quantitative oxidation.

ORGANIC COMPOUNDS AS REDUCTANTS

Organic matter has been described earlier as carbon in a state of reduction, the degree of this reduction dependent upon the character and quantity of other elements present. Oxidation returns these substituents to their original states. Consider a compound composed of the four primary organic elements: carbon, hydrogen, oxygen, and nitrogen. The products of complete reaction of this compound with dichromate in the procedure used here are CO_2, H_2O, and NH_3. Representing this compound with the amino acid glycine, the simple oxidative reaction would be

$$(1)\quad C_2H_5O_2N + 1\tfrac{1}{2}O_2 \rightarrow 2CO_2 + H_2O + NH_3.$$

The ammonia forms a salt in acid solution. Reaction kinetics of organic matter are not well understood, but the following balanced equation illustrates its general reaction with dichromate:

$$(2)\quad 2K_2Cr_2O_7 + 2C_2H_5O_2N + 9H_2SO_4 \rightarrow 4CO_2 + 10H_2O + (NH_4)_2SO_4 + 2Cr_2(SO_4)_3 + 2K_2SO_4.$$

Equation 2 serves only to demonstrate the stoichiometric relations. It is otherwise unnecessary to write a balanced equation for the dichromate reaction, and this operation becomes more difficult as molecular size increases. The relation of the reductant to oxygen is more easily seen in equation 1. One mole of glycine requires 1.5 moles of oxygen, and its Oxygen Equivalent in gram ratio is 48/75.1 or 0.639 grams of oxygen per gram of glycine.

The Oxygen Equivalent of any organic compound is most easily calculated by a simple valence-change method. Since oxidation-reduction is an electron-transfer process, the net change in valence which a reductant undergoes in a reaction indicates the amount of oxygen required (1 electron = 1 equivalent of oxygen). It is necessary to know, or to correctly assume, the valences of the elements before and after they have reacted. In the case of most inorganic reducing agents, the valences of the ions are obvious because of molecular polarity. Organic molecules, however, are bonded mainly by shared electrons (covalent bonds), and the assignment of valence to the elements becomes problematical. A practical method assigns the shared electrons to elements other than carbon according to the valence properties they display in normal inorganic states. Thus, oxygen is ordinarily negative and hydrogen is positive, and they are given the valences -2 and $+1$, respectively. Organic nitrogen, if present, is considered negative (amino, amide, etc.) and to it is ascribed a -3 valence. The effective valence of carbon in the molecule must therefore be a value which establishes electrical neutrality, or the sum of the valences of the other elements but opposite in sign. For glycine, the valence distribution is

$H_5 = +5$, $O_2 = -4$, $N = -3$, which total -2, and thus $C_2 = +2$.

This manner of assigning valence simplifies the interpretation of relations among the reaction products, for it is evident from equation 1 that the net valence change in the reaction is attributable to carbon alone. The total valence of carbon in the two moles of CO_2 produced is $+8$ (2 CO_2 contain 4 oxygens with a total valence of -8). The net valence change in the reaction is $+2 \rightarrow +8$, or 6 electrons per mole of glycine. The O.E. of glycine is $6 \times 8/75.1$, or 0.639 grams of oxygen per gram of glycine.

Calculation of the Oxygen Equivalent for a compound of known formula can be summarized in four steps:

1. Determine the effective valence of carbon in the compound by multiplying the number of atoms of each other ele-

ment by its assigned valence ($H = +1$, $O = -2$, $N = -3$) and summing these values with regard to sign. The carbon valence is the difference between the sum and zero.

2. Determine the valence of carbon as CO_2 by multiplying the number of C atoms by 4 (it will have a positive sign).

3. The difference between the valences of carbon in Steps 1 and 2 is the net valence or electron change of the reaction. It is equal to the equivalents of oxygen required (multiply by 8 to obtain the weight of oxygen).

4. The weight of oxygen divided by the molecular weight of the organic compound is its Oxygen Equivalent.

This method of calculating an O.E. applies to natural organic compounds wherein nitrogen carries a negative valence. Compounds containing nitrogen in a positive valence state require a revised valence assignment. Some compounds may contain elements other than those mentioned. Chloride is oxidized to free chlorine with a valence change of 1 electron. Other halides react similarly. Reduced sulfur is oxidized to SO_3 (or SO_4^{-2}). The maximum valence change for sulfur is +8, represented by the oxidation of sulfhydryl S (as in cysteine) to sulfate. It is necessary to add such valence changes to the net valence change of carbon when calculating the O.E. of a compound which contains these oxidizable elements. Some carbohydrate materials, such as agars and mucins, contain oxidized sulfur (as sulfamic groups or $-OSO_3-$ linkages) which requires no oxygen in reaction.

An Oxygen Equivalent may be calculated for a mixture of 2 or more compounds whose proportionate quantities are known by taking a weighted mean of their individual O.E.'s. It is also possible to determine the O.E. of organic matter from its ultimate composition by the method described above. The elemental percentages first must be converted into atomic ratios as they would appear in a hypothetical molecule. Assume that a sample of organic matter has the same ultimate composition as glycine: 32.0 percent C, 6.7 percent H, 42.7 percent O, 18.7 percent N. These percentages are divided by their respective atomic weights to give a hypothetical molecule whose weight is 100: $C_{2.66}H_{6.66}O_{2.66}N_{1.33}$. The valence change is found as outlined above:

$$\begin{array}{l}
H_{6.66} = +6.66 \\
O_{2.66} = -5.32 \\
N_{1.33} = -3.99 \\
\hline
C_{2.66} = +2.65 \rightarrow 2.66C \text{ (as } CO_2) \\
\qquad\quad = +10.64
\end{array}$$

The net valence change is $10.64 - 2.65 = 7.99$, and the weight of oxygen required is 7.99×8, or 63.92. The O.E. of the sample material is, then, $63.92/100 = 0.639$. This is a convenient way to determine the O.E. of a sample of unknown molecular composition, but whose elemental analysis is available. It presumes that none of the elements is present in inorganic combination.

There are two important assumptions concerning end products in the foregoing treatment which require a final qualification. The first is that all reduced nitrogen goes unoxidized and appears eventually as ammonium salts. There are no definite indications of nitrogen oxidation in the procedures employed here, which agrees with the observations of Williams (1937). However, Ark (1928) stated that imino nitrogen is oxidized by a sulfuric acid–dichromate mixture. The extent to which this might occur is not known. Nonoxidation of organic nitrogen does not hold true if prosphoric acid is included in the reaction mixture as suggested by Walkley (1947) and used by Allison (1960), Lawton (1955), and Mohlman and Edwards (1931). Christensen et al. (1937) found that the inclusion of phosphoric acid caused a nearly complete conversion of NH_3 to nitrate. The possible oxidation of some forms of organic nitrogen by the present dichromate procedure remains to be demonstrated. Should this occur, however, the overall effect on oxidative considerations of natural organic matter would be slight.

The second assumption to be qualified concerns incomplete oxidation which probably occurs to a limited extent in all reactions. Schollenberger (1945) indicated that some carbon monoxide may escape oxidation, and this phenomenon has been observed also by Christensen et al. (1937). However, it has not been noted in this study or by many other workers employing dichromate, even in "weak" partial oxidations. Since CO is quite readily oxidized, a thorough and prolonged mixing of the reactants as they are combined should preclude its evolution. Formic acid is a product of weak oxidation but apparently does not escape decomposition under strong oxidation potentials (Ark, 1928; Kuhn and L'Orsa, 1931). Other low molecular weight acids may be volatilized from a reaction mixture before they are decomposed. The only one to do this to an appreciable extent is acetic acid, which is quite resistant to oxidation and apparently is formed from ethoxyl or potential ethoxyl groups during the reaction (Kuhn and L'Orsa, 1931). The complete oxidation of acetic acid and other volatile unoxidized products is encouraged by catalysts and strong oxidation potentials. The effects of these factors will be considered later.

OXIDATION OF NATURALLY OCCURRING ORGANIC MATTER

The purpose of quantitative oxidation is to characterize organic matter from natural samples by its relation, in reaction, to oxygen. This fundamental relation is termed here, the Oxygen Equivalent. It is possible, in situations where the quantity of organic matter in a sample is known, to estimate an O.E. experimentally. More commonly, a sample is oxidized, and the resulting value is taken to represent, directly or by conversion, the amount of organic matter it contains. This oxidation parameter requires a term to distinguish it from the O.E., which is a distinct chemical property. It has been referred to in the literature in various ways: Chemical Oxygen Demand (C.O.D.), Oxygen Consumed, Oxygen Consuming Capacity, Oxidizability, Oxygen Demand, Oxygen Required, and Reducing Power. There is further confusion, especially in abbreviation, with expressions that refer to different parameters: Biochemical Oxygen Demand (B.O.D.), Dissolved Oxygen (D.O.), Oxygen Deficit, and the compound adjective, Oxidation-Reduction (O–R). To eliminate duplicate terminology and the possibility of confusion, the term Oxygen Consumed (O.C.) is proposed exclusively for the parameter in question.

These two oxidation terms can be defined, with special reference to natural organic matter, as follows:

The Oxygen Equivalent (O.E.) is the weight of oxygen required for complete oxidation of a unit weight of organic matter.

The Oxygen Consumed (O.C.) is the weight of oxygen required in the oxidation of any sample containing organic matter.

The O.E. is always a ratio, while the O.C. is simply a quantity of oxygen, as stated above. It is frequently desirable to express Oxygen Consumed as a ratio, that is, as the weight of oxygen required per unit weight, volume, or surface area of sample. The difference between O.C. and O.E. as weight ratios is in the distinction between true "organic matter" and "sample" (partly organic). These two values (as weight ratios) will be equal when the sample is purely organic.

Spoehr and Milner (1949) have used the expression "R-value," or "degree of reduction," in the oxidative characterization of algal matter. Briefly, it is definable as the quantity of oxygen required for the combustion of a unit weight of organic substrate, calculated on a 0–100 scale. The larger figure represents a maximum value for the most reduced compound (methane). The R-value concept includes the oxidation of organic N to N_2, but in other respects it is similar to the Oxygen Equivalent (for nonnitrogenous compounds, R-value $\times$ 0.04 = O.E.). The R-value does not refer to oxygen by weight directly (because it is a double ratio) and therefore lacks the utility of the O.E. The 0–100 scale, moreover, has little advantage in classifying organic matter over the comparable 0–4 O.E. scale.

In connection with the analysis of total organic matter in natural samples, it should be noted that the total organic substance, and not the carbon alone, is oxidized. Although the theoretical Oxygen Equivalent has been defined here in terms of carbon valence change, the initial carbon valence is ascribed to the other elements present, and thus they are directly involved. Juday and Birge (1932), in estimating the efficiency of their oxidative analyses in terms of carbon, stated that 2 atoms of oxygen (or 32 g.) are required per atom of carbon (12 g.). Although this is true for elemental carbon, it applies to organic carbon only in specific compounds (e.g., true carbohydrates) where it has an effective valence of zero. The oxidation of formic acid (CH_2O_2) and methane (CH_4) illustrates two divergent cases. One mole of each compound contains 1 gram atom (12 g.) of carbon; formic acid reacts with 1 gram atom (16 g.) of oxygen, and methane requires 4 gram atoms (64 g.) of oxygen. It is quite improbable that these researchers had samples with organic carbon existing at an exclusive, or even an average, valence state of zero (see the appendix). The point to consider is: while it is possible to interpret oxidation results in terms of carbon by assuming an average quantitative relation between C and O, it should not be assumed that a fundamental, invariable correlative exists between the quantity of oxygen required in a reaction and the amount of organic carbon present in a natural sample.

FACTORS AFFECTING THE EFFICIENCY OF DICHROMATE OXIDATION

Wet organic oxidation rates are finite; the rate and completeness of these reactions concern several factors whose effects are usually interdependent. It is necessary to study a procedure closely in order to adjust all possible conditions for maximum efficiency. The effects of these factors were either investigated by using the general procedure outlined previously or evaluated from literature reports. Since they do not operate independently, it was necessary to reinvestigate some factors in the light of later findings.

The oxidation potential: reagent concentration and temperature

A dichromate oxidation potential, in itself, does not express the rate of reaction; but for organic compounds in general, the higher the potential the more rapid will be the reaction. Reactant concentration and temperature are related in the simplified Nerst equation

$$E = E_o \frac{0.0001983\ T}{6} \log \frac{(H^+)^{14}\ (Cr_2O_7{}^{-2})}{(Cr^{+3})^2}$$

and can be considered together for convenience (see p. 6 for explanation of symbols).

Various reaction temperatures have been used in dichromate oxidations, the rate of reaction increasing with temperature. However, spontaneous decomposition of dichromate also increases with temperature, causing a definite loss in precision above about 150° C. Direct boiling methods require close adherence to a lower acid ratio (about 1:1) for this reason. Higher acid ratios may cause the mixture to explode if it is boiled directly. The temperature reached by a 2:1 mixture from heat of acid dilution (Allison, 1935; Walkley, 1935) is in the range of 115°-125° C., and is insufficient to oxidize all organic matter completely unless it is maintained by external heating. A boiling water bath continues the reaction with a negligible decomposition of dichromate.

Ames and Gaither (1914) are considered to be among the first investigators to get complete oxidation of soil organic matter with dichromate as a result of strong acid concentration (80-85 percent). Friedemann and Kendall (1929) studied the effect of water-acid relations on the oxidation of biological materials and clearly showed the superiority of reaction mixtures with low water contents. The situation has been reviewed more recently by Walkley (1947), who indicated a 2:1 volume ratio of concentrated H_2SO_4: aqueous dichromate. An optimal acid content is contingent, to a certain extent, on the procedure employed. Lower concentrations may achieve good results at high temperatures or with long reaction times. Higher acid concentrations precipitate dichromate (as chromic anhydride), nullifying the benefit of increased H-ion activity. The 2:1 acid:water ratio was adopted in this study. Precise measurement of acid is not necessary, but since H-ions are used in the reaction (also by the inorganic substances present) it is advisable to avoid falling below the specified amount of acid.

The effect of dichromate concentration on the oxidation potential is indicated in the Nernst equation (above). The initial concentration gives a specified potential that decreases as the reaction proceeds, according to the extent to which the dichromate becomes reduced. Consider first, the direct relation between the initial dichromate concentration and the effectiveness of oxidation as illustrated in figure 1. Three representative compounds were oxidized by the same procedure, except that the normality of the solution was varied. The concentration of reductant in each analysis was adjusted to react with 50 percent of the dichromate, assuming complete oxidation. Dichromate normality has been plotted logarithmically to stress the Nernst relation. It is evident that a practical lower limit of dichromate concentration must be selected in order to obtain reasonably good results. On the other hand, a maximum normality of 2.0 has been established in this procedure as the approximate solubility limit for dichromate in the reaction mixture. A dichromate normality of 0.05 has been chosen as the minimum for useful oxidation in the analyses described later.

Consider next the dichromate concentration at the end of the reaction. It has decreased in proportion to the amount of organic matter oxidized. Ideally, a constant dichromate concentration should be maintained throughout the reaction to expose all unoxidized organic matter to the same oxidation potential. On the other hand, the procedure must determine the change in dichromate concentration with reasonable accuracy and precision if the method is to be useful. Therefore, a sample of the proper size must be selected so that it reduces neither too much nor too little of the oxidant. These arbitrary limits are best decided by the particular objectives of the analysis. In general, one should strive for a reduction of about 50 percent of the dichromate. It may be permissible, in using 1.0 or 2.0 *N* dichromate, to reduce as much as 80 percent in a reaction. No more than 50

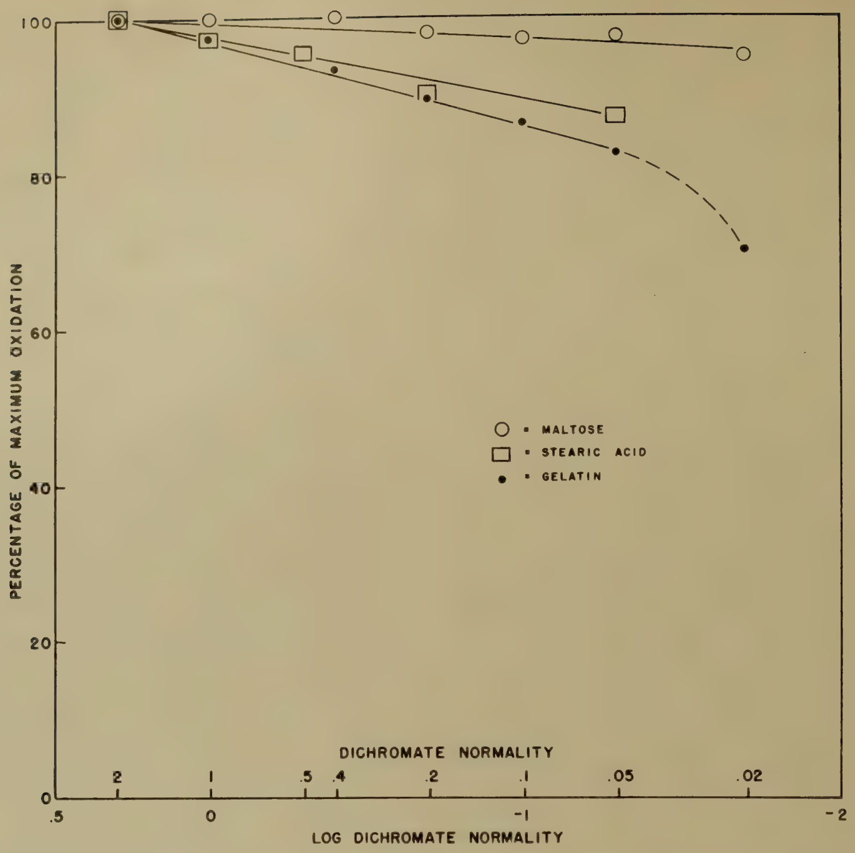

FIGURE 1.—Effect of different dichromate normalities, used in the same procedure, on the degree of oxidation of three organic substances.

percent of it should be reduced at low normalities, and acceptable results may be obtained, expecially when maximum possible oxidation is desired, with the reaction of only 5 percent of the dichromate present.

NATURE OF THE ORGANIC SUBSTRATE

Not all organic substances are oxidized with equal efficiency. Relative oxidation values for maltose, stearic acid, and gelatin, presented in figure 1, show that each of them reacts differently. This effect has been demonstrated for a wide variety of organic substrates, many of which are listed in table 2. These oxidations were accomplished over a period of many months by approximately the same procedure (the semimicro method described in part IV). Some of the earlier analyses, especially of amino acids, were made before the procedure had been fully revised and would probably give slightly higher recoveries if reanalyzed. However, the purpose of this tabulation is to show only the general differences in oxidizability among the various organic compounds.

There appears to be a distinct relation among the oxidative susceptibility, the reduction state, and the general biochemical character of the organic substances. They have been arranged according to these similarities in table 2, and can be described oxidatively in terms of the common proximate groups.

Group 1. — The "carbohydrates": strongly hydrated or hydroxylated com-

TABLE 2.—*Comparison of theoretical and experimental Oxygen Equivalents of various organic substrates*

Substrate	Oxygen Equipment		Percent recovery
	Theoretical	Experimental[1]	
Group 1:			
Maltose	1.065	1.06	100
Starch	1.184	1.18	100
Cellulose	1.184	1.18	100
Oxalic acid	0.127	0.126	99
Sodium oxalate	0.119	0.119	100
Tartaric acid	0.533	0.531	100
Ammonium tartrate	0.434	0.434	100
Group 2:			
Glycerol	1.126	1.16	95
Stearic acid	2.925	2.85	98
Palmitic acid	2.870	[2] 2.63	92
Corn oil	[3] 2.85	2.77	97
Group 3:			
Uric acid	0.285	0.290	102
Cteatinine	0.677	0.628	93
Creatine	0.848	0.77	91
Uracil	0.713	0.717	101
Asparagine	0.727	0.615	90
Cystine	1.131	1.07	95
Glutamic acid	0.979	0.972	99
Glycine	0.639	0.55	86
Leucine	1.825	1.58	87
Methionine	1.501	1.03	69
Tryptophan	1.801	1.82	101
Tyrosine	1.681	1.66	99
Gluten	1.56 [3]	1.44	93
Zein	1.62 [3]	1.45	90
Gelatin	1.33 [3]	1.20	90

[1] All values are means of 3 or more analyses by the semimicro method with precisions within ±5 percent.

[2] Technical grade, O.E. probably low because of impurities.

[3] Calculated from molecular compositions given by Spector (1956).

pounds that have a characteristic O.E. of 1.2 or lower and are completely oxidized by dichromate.

Group 2.—The lipids: compounds essentially of carbon and hydrogen that have a characteristic O.E. above 2.0 and are almost completely oxidized (95-100 percent) by dichromate.

Group 3.—The crude proteins: various nitrogenous compounds that have an aggregate O.E. of about 1.5 in natural organic mixtures and are incompletely oxidized (90-95 percent) by dichromate.

Most other prominent compounds can be classified into this scheme, and a more elaborate treatment is given in the appendix.

The incomplete oxidation of most of these compounds can be considered a function of either their chemical resistance or their physical state. Since all of the substrates do react, these factors must influence the rate of reaction. In order to be oxidized efficiently, the substrate must be maintained in intimate contact with the oxidizing solution. A physical state which reduces this contact, such as that in particles or immiscible fluids, will therefore hinder the reaction. Proteinaceous particles and fatty materials are common examples. Microzonal reduced areas form at their interfaces, and they tend to float or adhere to the surface of the glass, where contact with dichromate is further reduced. These effects can be offset in part by agitating the reaction mixture, and it is advisable to do this with all samples as they are combined, and intermittently during the digestion period.

The resistance of protein compounds to oxidation is also partly chemical. Cellulose particles, for instance, react rapidly and completely; conversely, a gelatin suspension reacts very slowly. Since many of the amino acids also exhibit this resistance, it seems to be most characteristic of group 3. Much of the incomplete oxidation can be explained by the formation of acetic acid, which is very difficult to oxidize. The composition and configuration of many proteinaceous molecules is such that they tend to produce acetic acid in the process of decomposing. This seems especially true of alanine, and probably also of leucine and other resistant amino acids. The oxidation of such substances can be improved by using a catalyst as discussed in the following section.

REACTION TIME AND CATALYSIS

All the foregoing factors are manifest in the reaction rate between dichromate and organic matter. Their combined effects are demonstrated in figure 2 as time-oxidation curves for the three substances representative of the proximate-oxidation groups described earlier. Identical procedures were used in the analyses ("Semimicro method"), and the sample sizes were adjusted to re-

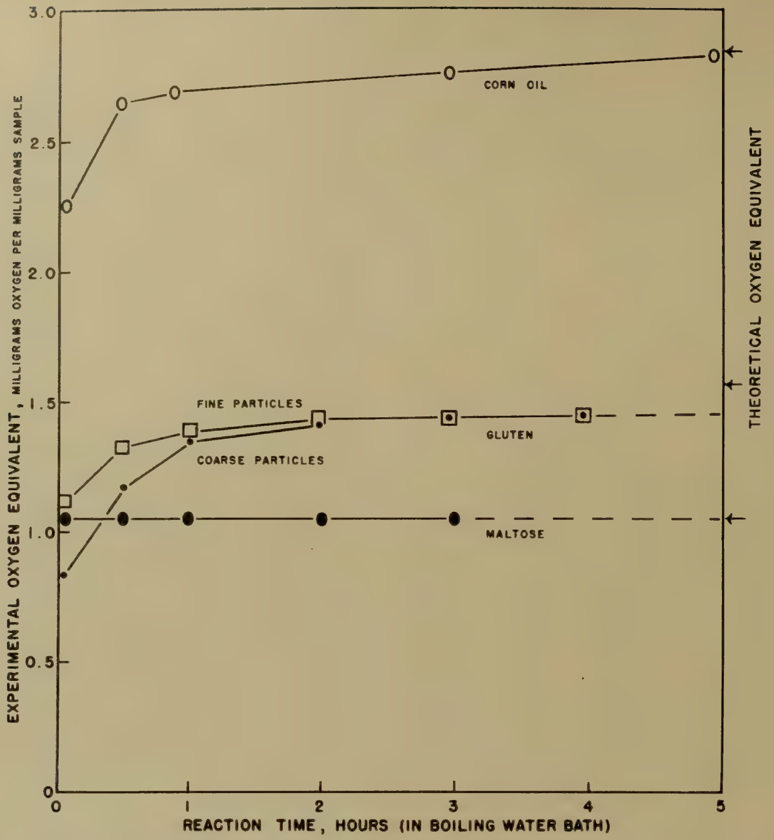

FIGURE 2.—Effect of reaction time on the completeness of oxidation of three organic substrates.

duce about 50 percent of the dichromate. Reaction time represents the number of hours each mixture was maintained in a boiling water bath. All of them were diluted and titrated at the end of 5 hours. Thus, at $t = 0$ the degree of oxidation is referrable to the reaction induced by heat of acid dilution, plus a minor amount of reaction which most probably occurred while the samples awaited analysis. In other words, if each sample had been diluted and titrated at the end of its specified reaction time, the curves would likely rise more sharply from a lower point on the ordinate.

Two curves are given (fig. 2) for gluten to show the influence of particle size on the rate of reaction. The sample material was roughly separated into two size groups by screening. "Fine gluten" was composed of particles about 0.2 mm. in diameter, and "coarse gluten" fragments ranged between 0.5 and 2.0 mm. The effect of surface area contact with the oxidizing solution is initially quite significant, but disappears at the end of about 2 hours' reaction time.

These curves can be considered characteristic of the oxidative response of the three proximate organic groups.

Group 1.—"Carbohydrates": The maltose curve is a straight line parallel to the X-axis at maximum theoretical O.E. Oxidation is complete with heat of dilution only and is not significantly affected by physical state because of strong oxidative susceptibility.

Group 2.—Lipids: The immiscibility of corn oil makes it difficult to oxidize. The reaction continues slowly with time and can be expected to reach completion eventually.

Group 3.—Crude proteins: Gluten is oxidized slowly and the curve levels off before maximum theoretical oxidation is attained. This effect is probably due to the loss of some unoxidized constituents.

From these data and other results not shown, an optimum reaction time of 3 hours in a boiling water bath has been selected. Although higher reaction temperatures can reduce this reaction time, the simplicity of a water bath and the reproducibility of results offset the temporal inconvenience. Incomplete oxidation, which is characteristic of certain compounds, is reproducible at any given dichromate concentration. It can be compensated for as an approximately constant error in any specified analysis.

A catalyst, silver sulfate, has been used in the preceding reactions, but its effect there has been slight. This is not true for all procedures. Lawton (1955) tested the efficacy of several metal ions as catalysts for dichromate oxidation using the Moore (reflux) technique. These findings indicated that silver was the most satisfactory catalyst, and was essential to the oxidation of acetic acid and its precursors. Many other researchers recommend its use. Christensen et al. (1937) and Van Slyke and Folch (1940), however, found catalysts ineffective in their oxidations. Friedemann and Kendall (1929) have pointed out the probable major cause of this discrepancy. They found that acetic acid was completely oxidized by a 1:1 ratio of acid:dichromate solution in the presence of Ag^+, but only 50 percent oxidized in its absence. Using the same procedure with 70-percent acid, they were able to oxidize acetic acid effectively without the catalyst. Thus, the catalytic effect of silver becomes less evident as the oxidizing conditions are made more rigorous. In the procedure employed here, silver sulfate increases the recovery of difficultly oxidizable substances by a few percent, at most. Considering that it produces no inimical effects in a strictly oxidative analysis, silver sulfate may well be included in the reaction mixture. It is conveniently added as a solute in the acid used.

Interferences

A number of other factors which operate to increase or decrease the observed Oxygen Equivalent from its true value are classified together here as interferences. They may be severe in special instances, but usually can be avoided or compensated in the final analysis.

Oxidizable inorganic substances can give erroneously high results if not properly regarded. A sample taken from an aerobic situation is not likely to contain dichromate-oxidizable inorganic compounds with the exception of halides, chlorides in particular. Chloride is oxidized to free chlorine, and the presence of even minute amounts in a reacting mixture is detectable from the odor of Cl_2 at the mouth of the flask. This reaction is not rapid, and is complicated by the formation of chromium oxychloride (CrO_2Cl_2), which is volatile and may be observed as a dark-red liquid adhering to the upper, inner surfaces of the reaction flask. Soluble chlorides are precipitated as AgCl if silver sulfate is included in the reagents. Some reports imply that chloride is not oxidized from the silver precipitate (Kay, 1954; Nikolaeva, 1953; Peech et al., 1947), but others indicate that it is at least partly oxidized (A.P.H.A., 1956; Buydens and Ledent, 1957). The opposing views apparently result from different procedural effects.

The oxidizability of chloride as Cl^- and AgCl was tested by the procedure employed in this study. After 1 hour's reaction time, 73 percent of the dissolved chloride and 68 percent of the precipitate had been oxidized. At the end of 3 hours, nearly all of the AgCl was oxidized, but the Cl^- recovery increased only slightly (perhaps owing to retention of undecomposed chromium oxychloride). A further observation was that Ag_2SO_4 seemed to preclude the formation of CrO_2Cl_2, which would be a benefit additional to its catalytic function.

The presence of small amounts of chloride in a sample (about one-tenth the weight of organic matter) usually can be disregarded because the Oxygen Equivalent of chloride (0.226) is less than one-sixth that of "average" organic matter. A sample Oxygen Consumed value can be corrected for moderate amounts of chloride by quantitatively analyzing for Cl^- in a duplicate sample aliquot and subtracting the calculated chloride O.C. (chloride O.E. × mg. present) from the total O.C. If the chloride O.C. exceeds the organic O.C. in a sample, other methods of adjustment will be necessary in order to obtain a reasonably accurate quantitative organic oxidation.

A sample from an anaerobic milieu can be expected to contain other oxidizable inorganic elements, especially ferrous iron and reduced sulfur. These may be serious interferences, for instance, in samples of lake muds. Reduced inorganic elements become oxidized in contact with atmospheric oxygen. This might occur sufficiently during sample treatment (drying, grinding, storage) to remove the interference. Possibly, the sample could be preoxidized with a mild reagent to convert the reduced inorganic matter before quantitatively oxidizing it with dichromate. A confounding factor is the presence of unstable organic compounds which partly oxidize in contact with the atmosphere. The quantitative oxidation of samples from highly reduced environments appears to be feasible, but the correct interpretation of such results requires considerations beyond the present treatment.

The most common analytical interference in a quantitative oxidation is the presence of contaminants. The general precautions of microanalysis are applicable here, especially in regard to reagent purity and cleanliness of glassware. Reaction flasks should be cleaned with chromic acid and kept covered to avoid direct contact with the atmosphere. For critical studies, special redistilled water (Medalia, 1951) and recrystallized dichromate (Kolthoff and Sandell, 1952) are advised. The reagent blank compensates for some, but not all, contamination that may occur.

The loss of unoxidized substances and the spontaneous decomposition of dichromate are two interferences that have been mentioned previously. A reflux condenser can eliminate the loss of most volatile matter, but it also complicates the procedure. The use of a suitable reaction flask minimizes such losses; Kjehldahl flasks are excellent in this respect, but cannot be used with all types of samples. Regardless of the kind of flasks used, they should be immersed in the water bath only at the base, so that the upper portions remain cool and permit condensation to occur. Spontaneous decomposition of dichromate cannot be measured independently from reduction by contaminants in the reagents but appears to be negligible in this procedure. The total loss of dichromate in the absence of an intentional reductant increases logarithmically with reaction time, as evidenced by reagent blank titers. The observed magnitude of this loss in a reaction period of 3 hours is usually less than 2 percent with 1-2 *N* dichromate and less than 5 percent with 0.05 *N* dichromate. If losses much higher than these are encountered, the analyses should be viewed with suspicion.

Part IV. METHODS OF DICHROMATE OXIDATIVE ANALYSIS

The diverse nature of limnological samples and their wide range of organic concentrations each impose special methodological considerations. First, the general procedure requires a sample of a certain physical state, and the validity of analysis requires that the organic matter in the sample be as little changed as possible from its original state. The treatment given to a sample before analysis, therefore, is no less important than the analysis itself. Second, the general procedure was selected partly because of its usefulness over a broad analytical range. To accomplish this effectively for a variety of samples, a few minor procedural adjustments are necessary. Two specific methods of oxidation are described below which apply to the most common sample types: particulate matter that is available in weighable amounts, and small quantities of dissolved substances that are meted in terms of water volumes. Analytical adjustments necessary for the extension of these methods to samples of other types and sizes are suggested.

PREANALYTICAL SAMPLE TREATMENT

The technique of oxidative analysis actually begins with sampling, but a discourse on this subject is beyond the present objectives. One word of caution is offered: the results obtained by oxidative analysis will be no better than the care and consideration given to the taking of representative samples. The following discussion, then, refers to samples which have already been secured. It is convenient, although somewhat factitious, to consider the initial form of a sample as either a weighable particulate mass or material dispersed in a large quantity of water. The former refers to such as filter or centrifuge residues, sediments, periphyton, benthic fauna, and even macrophyte or vertebrate tissues; the latter concerns only water samples, with or without dispersed particulate matter.

Solid materials usually are analyzed on a weight basis, but sometimes a volume (e.g., plankton), surface area (e.g., periphyton) or numerical (e.g., individual invertebrates) basis is preferable, especially if the sample is analyzed fresh. Samples intended for later analysis are conveniently preserved by drying, which is best done at low temperatures in a vacuum. Lyophilization probably is a better method. Dried samples frequently require grinding in a mortar to reduce them to fine particles; this process also increases homogeneity. A dried, ground sample should be stored in a closed vial.

An Oxygen Consumed value can be determined for any sample by quantitative oxidation, as previously discussed, but an Oxygen Equivalent can be estimated analytically only if the organic weight of a sample is known with reasonable accuracy. Obtaining the O.E. of a weighable sample is an important consideration, for it permits a better characterization of the organic matter present. Natural samples contain mineral matter and water in addition to organic compounds, and the proportionate weight of these inorganic constituents can be determined for many samples by careful drying and ignition. Organic matter is notoriously hygroscopic; analysis on an air-dry basis alone is not always satisfactory, even for comparative oxidations. Birge and Juday (1922) described the difficulty of removing residual moisture from plankton material and found that dried samples stored in bottles for prolonged periods lost up to 7 percent of their weight upon redrying. Similar experiences were encountered in this study (1 to 3 percent moisture gain in vacuum-dried samples during storage for several months), and it was considered necessary to redry all stored samples at the time of analysis. Probably it is impossible to remove all traces of water from organic matter; the moisture content is merely reduced to an insignificant level. Determination of the mineral content of moisture-free material by ashing is subject to error (see p. 878 in Hutchinson, 1957) and can be used in the approximation of true organic content only when the proportion of mineral in the sample is relatively low. The ash content of samples reported here was measured by igniting the weighed dry samples at about 600° C. for a few hours, rehydrating the cooled residues with a few drops of distilled water, redrying at 100°-105° C., and weighing them again after they had cooled in a desiccator.

The treatment of water samples presents a different sort of problem. Efficient oxidation depends on a low water content in the reacting mixture. Except in unusual cases of high organic content, water samples must be concentrated for analysis. Ordinarily, between sampling and concentration there is a significant time lapse during which oxidative decomposition might occur. Oligotrophic lake waters, for instance, may contain more than twice the amount of free oxygen needed to oxidize all of the organic matter they contain. Skopintsev (1949b) has observed that the organic content of untreated river water decreases logarithmically during storage at 16°-20° C. The initially rapid rate of loss, attributed to labile components, became slower and nearly constant after 40 to 50 days. Not all such oxidative decomposition is microbial. Krogh and Lange (1932) found that dark-stored lake water, apparently free of microorganisms, consumed a few milligrams of oxygen per liter during the course of a month. Autolytic transformations in bacteria-free algal sediments were studied by Aleyev (1934), who demonstrated that lysis proceeds slowly but the causative enzymes retain their activity for a long time. Ultraviolet radiation also promotes the reaction between dissolved oxygen and organic matter (Drachev and Mityagina, 1957).

Organic matter from waters normally saturated with oxygen may be more stable than that indicated above. In this study, there was no detectable change in the organic content of oligotrophic lake water stored dark, sealed, and refrigerated for periods up to a few weeks. Preventive treatment may be necessary for labile samples or for prolonged storage. Frozen storage or removal of free oxygen (as by flushing with N_2 or CO_2) might prove satisfactory. Acid fixation in the field, as is frequently used in marine research (Duursma, 1960; Kay, 1957), is another possible method. Organic fixatives, such as toluene or chloroform, should not be used since they are never removed completely from the sample, even when it is evaporated to dryness. The oxidative decomposition of organic matter in water requires further study; the safest practice to follow in the quantitative oxidation of water samples is to analyze them as soon as possible after they have been taken (see Strickland and Parsons, 1960, p. 113).

A water sample is most frequently concentrated for analysis by evaporation in an oven at 100°-105° C. (e.g., Krogh and Keys, 1934). Nikolaeva (1953), however, selected 60°-70° C. as optimum for evaporation and also neutralized the water with sodium carbonate to prevent the loss of volatile acids. In the present investigation, 50–250 ml. aliquots of water were evaporated from widemouth Erlenmeyer flasks, which also served as oxidation vessels. The effect of evaporation temperature on Oxygen Consumed values of oligotrophic lake water was tested. Triplicate portions of two samples, one filtered (millipore HA) and one unfiltered, were evaporated to dryness in a thermostatically controlled electric oven at various temperatures. The data are shown in figure 3. Temperature of oven operation ranged within ±3 degrees of the selected value. The difference in relative displacement of the two curves indicates the O.C. of seston contained in the unfiltered sample. The shapes of the curves suggest a difference in response to drying temperature. Unfiltered water residues consumed most oxygen after drying at about 90° C., but the residues of filtered water dried at 78° C. showed the greatest response to oxidation. These findings indicate an optimum drying temperature range for this water of 80°-90° C. The effect of drying temperature on maximum organic retention is not known for other waters but is expected to vary.

Concentration of water samples by drying at elevated temperatures is obviously unsatisfactory for critical studies because of the uncertain losses and probable chemical changes of the organic matter. Drying at intermediate temperatures requires a long time, during which enzymatic oxidations evidently occur. Freeze-drying probably is most efficacious but requires special apparatus and is difficult to apply to large volumes of water. Freeze-concentration may prove to be a very useful technique for studies concerning dissolved and dispersed organic matter.[1]

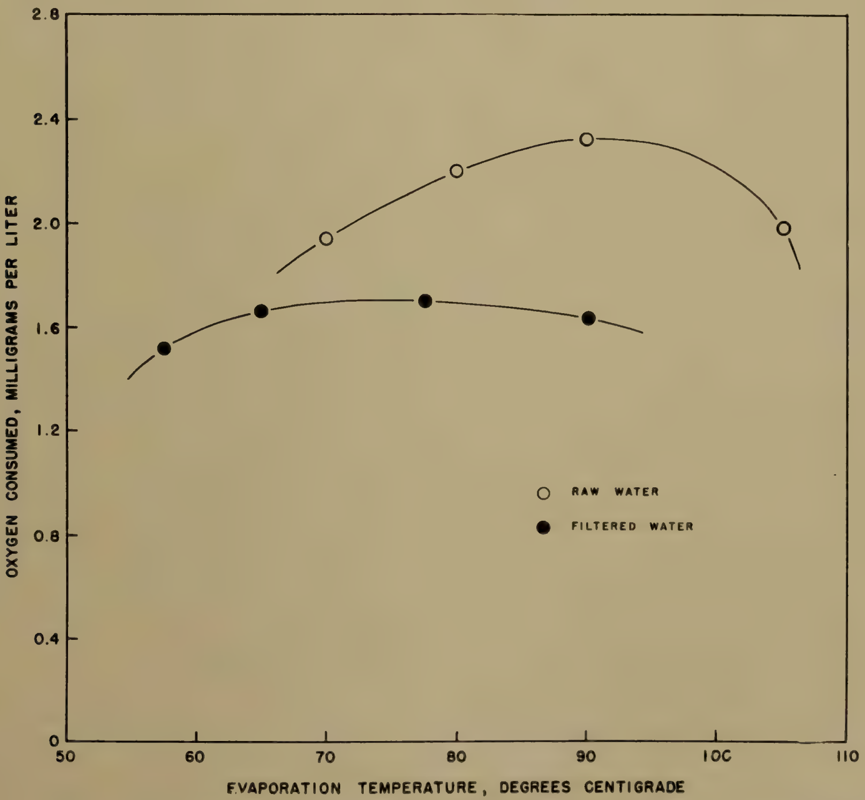

FIGURE 3.—Effect of evaporation temperature on the oxygen consumed by raw and filtered lake-water samples.

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[1] Simple, slow freezing is effective in concentrating the solutes from water. An 8-liter sample of filtered pond water was slowly reduced to 1 liter of liquid in a deep-freeze unit. The separated phases gave the following evaporation (T.D.S.) data: Liquid, 542 mg./L.; ice, 6 mg./L. See also pages 98–99 in Ellis et al. (1948), page 170 in Welch (1952), and Shapiro (1961).

Semimicro method

A technique was needed at first to investigate the oxidizability of purified organic compounds, and later to characterize the particulate matter (e.g., plankton) from the aquatic environment. Its principal requirement was that it be applicable to the smallest samples accurately weighable on a standard analytical balance. The following categorical procedure concerns a dry sample requiring about 40 mg. of oxygen for complete oxidation (which represents about 25–30 mg. dry, ash-free biological material). However, the method is broadly applicable to samples that are dry, moist, or in solution, and which may consume from about 2 to 100 mg. of oxygen (about 2–70 mg. natural organic matter). The extended range of analysis is accomplished by minor alterations of reagent volume or concentration which are discussed subsequently.

1. Reagents:

a. Standard potassium dichromate, 2 *N*: Dry 100 g. reagent grade $K_2Cr_2O_7$ crystals at 150° C. for 2 to 3 hours, cool in a desiccator, weigh out and dissolve 98.07 g. in distilled water, and make up to 1 liter.

b. Ferrous sulfate, approximately 1 *N*: Dissolve about 280 g. $FeSO_4 \cdot 7H_2O$ in distilled water, add 20 ml. concentrated H_2SO_4 and make up to 1 liter. It is advisable to filter this solution and store it in a stoppered brown-glass bottle. Standardize, when used, against the dichromate solution. One-half normal ferrous ammonium sulfate can be employed alternatively, but higher normalities may result in a supersaturated solution from which the salt precipitates during storage.

c. Sulfuric acid, concentrated (96 percent or more).

d. Phosphoric acid (ortho-85 percent).

e. Silver sulfate: Dissolve 12 g. Ag_2SO_4 in each liter of H_2SO_4.

f. Barium diphenylamine sulfonate (indicator): Prepare a 0.3-percent solution by dissolving 0.15 g. in 50 ml. warm distilled water.

2. Glassware:

a. Reaction flasks: 30 ml. Kjehldahl or round-bottom boiling flasks of a similar size.

b. Titration flasks: 250 ml. Erlenmeyer.

c. Volumetric ware: one transfer pipette, 5 ml.; one burette, 10 ml. (semimicro), calibrated at least to 0.05 ml.; two graduated cylinders, 10 ml. and 200 ml.

3. Procedure:

It is expeditious to oxidize a series of samples or sample replicates simultaneously. At least one reagent blank should accompany each series through the entire operation (steps b through g). The following procedure applies to any member of the series.

a. Weigh out a sample which will require 20–50 mg. oxygen (e.g., about 30 mg. dry algal material) and place it in the bottom of a reaction flask. This may be done conveniently with a long-shanked weighing scoop (see Van Slyke and Folch, 1940) or by weighing the sample into a small, clean glass cup which can be admitted and left in the reaction flask.

b. Pipette 5 ml. standard dichromate into the flask, allowing the solution to run down the inner surfaces of the neck as the flask is rotated. Swirl gently to disperse the sample in the dichromate.

c. Measure about 11 ml. concentrated sulfuric acid into a graduated cylinder (1 ml. excess is allowed here for the acid which adheres to the sides of the cylinder). Add rapidly to the reaction flask while swirling it gently. The reaction progresses immediately upon acidification, and swirling should be continued for about 30 seconds.

d. Stopper the flask lightly with a glass or polyethylene plug and immerse the base in a boiling water bath so that its neck is cooled by ambient air. Note well: This mixture should not be boiled directly nor exposed to a higher temperature for prolonged periods.

e. Maintain the flask in the bath for 3 hours, swirling it at about half-hour intervals. There should be no organic particles observable in the flask after 2 hours. Before the final swirling, hold the flask at a steep angle and rotate it to rinse the lower part of the neck with the oxidizing mixture. Remove the flask from the bath after 3 hours and cool slightly.

f. Transfer the contents to a titration flask, rinsing it out with several successive portions of distilled water. Dilute the mixture to about 150 ml.

g. Cool the diluted solution to room temperature, add 5 to 10 ml. phosphoric acid and 2 or 3 drops of indicator. Titrate with the ferrous solution. The endpoint is very sharp and reversible, changing from a red or reddish blue to bright green. The reagent blank will require about 10 ml. 1 *N* ferrous solution and the samples proportionately less, according to organic content.

h. Since the ferrous solution requires standardization, it is conveniently done in duplicate at the time samples are titrated. Pipette two 5-ml. aliquots of 2 *N* dichromate into clean titration flasks, add about 150 ml. distilled water and 5 ml. sulfuric acid to each, mix, cool, add 10 ml. phosphoric acid and 2 drops indicator. Titrate each as described above.

4. Calculations:

a. Standardization of reductant:

$$\text{Normality Fe}^{+2} = \frac{\text{ml. dichromate} \times \text{normality dichromate}}{\text{ml. Fe}^{+2}\text{ solution}}$$

b. Oxygen Consumed: O.C. = (reagent blank titer − sample titer) × *N* Fe^{+2} × 8, with titer volumes in milliliters. This equation gives the milligrams of oxygen the sample required; divide by the sample weight (mg.) to express O.C. as a weight ratio.

c. Oxygen Equivalent: If the sample is purely organic, the O.C. (as weight ratio) represents the experimental O.E.; if it is partly organic, an experimental O.E. is calculated on a moisture- and ash-free basis. Exper. O.E. =

$$\frac{100 \times \text{O.C. (as wt. ratio)}}{100 - (\%\text{ moisture} + \%\text{ ash})}.$$

5. Example:

Samples of three plankton organisms were oxidized by the semimicro method, and this data is presented in table 3. The two genera of phytoplankton and one of zooplankton were obtained in a relatively pure state from Crowley Lake, Calif., by screen filtrations of localized concentrations. The analysis was done in replicate in two series. The only departure from the outlined procedure was the use of 1 *N* dichromate (Fe^{+2} normality and weights analyzed were proportionately lower). The precision of the replicates, expressed as both coefficient of variation and percentage average deviation, is generally within ±1 percent. The higher coefficient of variation for *Aphanizomenon* is probably caused by heterogeneous sample material, since it was not ground as fine as the other two substrates. The interpretation of these oxidation values will be discussed later.

6. Comments:

To extend this method over a wider range of sample sizes without significantly changing the effectiveness of oxidation requires only slight modification. Samples needing up to 100 mg. oxygen (or containing up to 80 mg. organic matter) can be analyzed by doubling the reagent volumes and by using 100-

TABLE 3.—*Oxidation analyses of plankton by the semimicro method*

Series	Sample		Ferrous solution, milliliters[1]		Oxygen Consumed, milligrams	
	Plankton organism	Air-dry weight (mg.)	Titer	Sample equivalent	Per sample	Per milligram of sample
A - 1	Reagent blank	0	9.76			
2	do	0	9.76			
3	*Aphanizomenon*	16.0	4.86	4.90	19.90	1.244
4	do	21.1	3.41	6.35	25.79	1.222
5	do	18.9	4.05	5.71	23.19	1.227
6	*Daphnia*	13.5	6.26	3.50	14.21	1.052
7	do	21.6	4.27	5.49	22.30	1.032
8	*Gleotrichia*	17.3	4.60	5.16	20.96	1.212
B - 1	Reagent blank	0	9.81			
2	do	0	9.81			
3	*Daphnia*	18.4	5.15	4.66	18.89	1.027
4	do	18.1	5.22	4.59	18.61	1.028
5	*Aphanizomenon*	22.6	3.14	6.67	27.04	1.196
6	do	18.9	4.12	5.69	23.07	1.221
7	*Gloeotrichia*	19.3	4.04	5.77	23.39	1.212
8	do	20.1	3.81	6.00	24.32	1.210

Analytical summary:		*Aphanizomenon*	*Daphnia*	*Gloeotrichia*
Oxygen Consumed:				
Mean value		1.22	1.04	1.21
Average deviation	percent	±0.9	±0.8	±0.1
Coefficient of variation	percent	±1.4	±1.0	±0.1
Inorganic content:				
Ash	percent	4.7	19.4	4.0
Moisture	percent	4.2	6.3	5.5
Oxygen Equivalent		1.41	1.46	1.41

[1] Normality = 0.507.

ml. Kjehldahl flasks. Dilution for titration must be increased correspondingly. The analytical range can be lowered similarly by decreasing reagent volumes and flask sizes, or by using 1 *N* dichromate (as in the above example) which is minimum for good efficiency and precision. A sample containing about 2 mg. organic matter can be analyzed satisfactorily using 1 ml. of 1 *N* dichromate and 2 ml. acid in a test tube.

This method is not restricted to dry samples. Moist or aqueous material of suitable organic content can be analyzed if attention is given to the minimum dichromate normality and proper acid: water ratio (2:1). To oxidize an aqueous sample, dichromate can be used in a dry state (e.g., pipette the required amount of standard dichromate into an empty reaction flask and evaporate to dryness before adding the sample solution). The precision shown in the preceding example is about average for natural samples. Pure organic compounds can be oxidized by this method to within ±0.5 percent of the mean value, if the procedure is followed with care (cf., exper. O.E.'s. in table 5). The reagent blank titer should be within 3 percent of the standardization titer.

Micro method

The following method was designed to analyze samples of natural waters, especially those which are low in organic content. It is actually only a variation of the preceding method, differing in strength of reagents and in sample manipulation. The procedure outlined is optimal for samples requiring slightly less than 1 mg. oxygen (or, samples containing from 0.07 to 0.7 mg. organic matter). However, it can be used on natural waters throughout their range of organic concentration without procedural alteration because the sample aliquot is easily adjusted to optimum reaction level.

1. Reagents:

a. Standard potassium dichromate, 0.05 *N*: Prepare by diluting 2 *N* dichromate, or dissolve 2.452 g. dry, reagent grade $K_2Cr_2O_7$ in distilled water and make up to 1 liter.

b. Ferrous sulfate, approximately 0.03 *N*: Either dilute the 1 *N* solution and acidify to 2 percent H_2SO_4 by volume, or dissolve 8.4 g. $FeSO_4 \cdot 7H_2O$ in distilled water, add 20 ml. concentrated H_2SO_4, cool, and dilute to 1 liter.

c. Other reagents as in the semimicro method: Concentrated sulfuric acid with silver sulfate, concentrated phosphoric acid, barium diphenylamine sulfonate indicator.

2. Glassware:

a. Combined evaporation-reaction-titration vessels: 250 ml. widemouth Erlenmeyer flasks with glass or polyethylene covers.

b. Volumetric ware: All items listed in the semimicro method, plus two transfer pipettes, 50 and 100 ml.

3. Procedure:

It is advantageous to treat a series of four or more samples concurrently. The following operations describe a single member of the series. At least one reagent blank, for which step a is omitted, must be included with the series.

a. Pipette an exact volume of sample water, which will require from 0.1 to 1 mg. oxygen, into an evaporation flask. Normally, this will be between 50 and 100 ml., and is conveniently considered in 50-ml. steps. If the approximate oxygen requirement of the water is unknown, use a 50-ml. aliquot in a pilot analysis and adjust to an optimum level, if necessary, in subsequent analyses. Evaporate the sample to dryness at no more than 100° C. (see page 19), taking precautions against contamination from the atmosphere.

b. Proceed exactly as in steps b through e, g, and h of the semimicro method, except use the reagents specified here. The reacted mixture is not transferred to a titration flask. Instead of semimicro step f, remove flasks from the bath and dilute contents in situ with 100 ml. distilled water.

4. Calculations:

a. Standardization of ferrous solution: Use formula in semimicro method.

b. Oxygen Consumed: Mg. O.C. is calculated as in semimicro method. Since the samples here are analyzed on a volume basis, the expression of O.C. as a ratio is calculated on a liter basis. O.C.

$$\text{in mg/L.} = \frac{\text{O.C. in mg.} \times 1000}{\text{ml. sample used}}.$$

5. Example:

Table 4 contains the results of analyses of 1 filtered and 2 unfiltered oligotrophic lake water samples, 4 determinations (of 200 ml. aliquots) having been made on each. Raw mean water and Filtered mean water are from the same original sample. The difference in their O.C. values is the O.C. of the seston. In general, the precision shown is about ±4 percent as the average deviation, and about ±5.5 percent as the coefficient of variation.

6. Comments:

Using a pure, easily oxidized compound such as sugar for a substrate, a precision of better than ±2 percent is attainable. Lower precision for raw water is a common phenomenon apparently due to chance occurrence of from none to a few macrozooplankters in any given aliquot. One aliquot without a *Daphnia,* for instance, may yield an O.C. of 0.5 mg/L. A second aliquot, identical to the first except for the presence of two 2-mm. *Daphnia* (with eggs, dry weight = 0.12 mg.; Richman, 1958), would have an O.C. of 0.62 mg/L. (based on data of table 3), or differ by more than 20 percent. Several replicate analyses may be needed to reach a valid mean O.C. in such cases. For this method, reagent blank titers should be within 5 percent of standardization titers. If they differ more, the procedure should be examined for possible sources of contamination.

TABLE 4.—*Quantitative oxidation of oligotrophic lake water*[1] *by the Micro Method*

Aliquot	Oxygen Consumed, milligrams					
	Raw mean water [2]		Filtered mean water [2]		Raw mean-depth water	
	Per aliquot	Per liter	Per aliquot	Per liter	Per aliquot	Per liter
No. 1	0.566	2.83	0.500	2.50	0.614	3.07
No. 2	0.644	3.22	0.560	2.80	0.524	2.62
No. 3	0.610	3.05	0.550	2.75	0.568	2.84
No. 4	0.600	3.00	0.538	2.69	0.578	2.89
Mean Oxygen Consumed		3.03		2.69		2.86
Average deviation		±3.7%		±3.4%		±4.4%
Coefficient of variation		±5.4%		±4.9%		±6.5%

[1] Convict Lake, Calif., 9 July 1959. Mean depth 52 feet. All analyses were made by use of 200-milliliter aliquots.
[2] A mixture of equal water volumes taken from 5 strata selected to represent the lake average.

It is difficult to increase the sensitivity of this particular method for two reasons: First, lowering the dichromate normality is not advised because of reduced oxidative efficiency (see part III); second, the volume of the reaction mixture needed to cover the inner surfaces of the flask adequately is about 12 ml. (4 ml. Cr^{+6}, 8 ml. acid), below which recoveries become too devious. However, a simple micro method, patterned after the suggestion on page 22 but using 0.05 *N* dichromate, can be devised for special samples (particles or solutions up to 2 ml. in volume). A single *Daphnia*, as described above, can be quantitatively oxidized by 1 ml. of 0.05 *N* dichromate and 2 ml. acid in a small reaction tube. The micro method can accommodate larger amounts of organic matter than is stated, by employing more concentrated solutions of dichromate. If water contains more than 40 mg. organic matter per liter, a convenient approach would be to evaporate the dichromate in a reaction flask (see page 22), add enough sample water to reconstitute a 0.05 *N* solution, and acidify at the 2:1 ratio.

Discussion

The methods presented here are proposed not as limnological standards but rather as introductory approaches to a broader investigation of natural organic matter. Their function in limnological research will become more apparent in the following treatment. The immediate concern is the improvement in the quantitative oxidation technique, especially in regard to time and completeness of oxidation. Moreover, the basic procedure may not fit the needs of other particular problems. To this end, some final methodological explanations may be of assistance.

1. Reagents and titration:

Dichromate solutions are extremely stable until reacted and require only normal care in handling. Ferrous solutions are rather unstable under some conditions. They are acidified (about 2 percent acid by volume) to increase stability and storage life. Actinic and temperature factors also affect stability but if ferrous solutions are stored in stoppered, brown-glass bottles in a cool place, their titer loss after a couple of months amounts to less than 10 percent. When used daily in the same manner, the normality of ferrous solutions was observed to decrease at a constant rate (about 0.5 percent per day). This suggests that daily standardization may not be necessary in an uninterrupted series of analyses.

Titration indicators are organic compounds and are susceptible to decomposition by strong acid and dichromate concentrations, and even weaker oxidizing solutions at high temperatures. Thus, it is necessary to dilute and cool the reaction mixture. Dilution also serves to reduce the color intensity of dichromate-chromic ions, giving the requisite transparency for titration. Phosphoric acid is added to complex ferric ions formed during the titration, thus lowering the ferrous-ferric system potential to give a better endpoint with the diphenylamine sulfonate indicator. The color change at the endpoint is complex because the color observed at any point is the result of both indicator and chromium compounds. The exact shade varies with dilution and relative concentrations of the color-producing ions. Chromium ions change from orange to bright green through continuous transitional intermediates. Superimposed is the indicator color, a reddish violet in the oxidized state and colorless when reduced. The endpoint change is always sudden and sharp, even in dilute solutions as a few trial titrations will demonstrate. Back titration is possible but not necessary after a little experience is gained. If the endpoint is consistently overshot, the analyst is advised to reserve a few milliters of the untitrated mixture in a clean container, rinse this into the titration flask when the initial endpoint is exceeded, and proceed cautiously to the final endpoint.

There are other satisfactory indicators for this titration which are discussed in analytical texts (e.g., Kolthoff and Sandell, 1952). The excess dichromate also can be determined colorimetrically by measuring the absorbance of the reaction mixture at 645 millimicrons and comparing it to a standard absorption curve (for details, see Graham, 1948).

2. Glassware and other equipment:

The glassware listed above is considered most suitable for these methods, but other items may be substituted or added to the list. The only essential piece of volumetric ware for austere circumstances is the burette. It may be used first to deliver the necessary volume of dichromate, rinsed, and then employed in the titration. Test tubes or small beakers, calibrated with the burette, can be used to measure out the acids.

Accessions which can expedite a large number of concurrent analyses are a reservoir burette and a magnetic stirrer. The latter is especially functional since adept endpoint detection depends to a large extent on how well the solution being titrated is kept mixed.

Inner surfaces of the pipette or burette used to dispense the dichromate solution become "dry" after a short time and require cleaning. This can be done by flushing the item with dilute hydroxide, followed by a rinse with distilled water.

3. Analytical precision and accuracy:

Both methods are capable of higher precision than shown in the examples, by using pure compounds as reductants and by taking special care in the preparation and measurement of reagents. It seems pointless to demonstrate this, however, since these methods are intended for the analyses of natural samples whose organic heterogeneities usually impose separate limits on the precision attainable. Samples of filtered water frequently replicate with high precision, but this is not always true. The drying process apparently exerts a major influence on reproducibility. Samples used in examples above were selected as representative of the limnological material to which these methods are applicable, and the stated precisions (semimicro method = ±1 percent; micro method = ±5 percent) are considered to be of a corresponding representative order of magnitude.

The accuracy of these methods on natural substrates is difficult to assess. It was pointed out in part III that some compounds are completely oxidized, while others give various incomplete recoveries (see table 2 and figs. 1 and 2). For any specific compound, the proportion unoxidized can be considered an almost constant error and therefore can be adjusted. Since there are a great many organic compounds in a natural sample, certain assumptions are necessary in adjusting its observed oxygen requirement to a "true" value. This has been done through the medium of proximate groups, which are discussed in the appendix. It is presently assumed that the oxidation of natural organic matter is 95 percent complete by the semimicro method, and 85 percent complete by the micro method. In using either method, directly or with modification, it is advisable to check the accuracy occasionally by means of "standard reductants". The absolute accuracy is best measured by using primary standard grade sodium oxalate. A convenient relative standard having oxidative stability is gelatin, which can be weighed in a dried form or pipetted from a "solution" of known concentration.

The presence of inorganic chloride in a sample will also affect the accuracy, and corrections must be made in the final results. It has already been suggested that, at interfering levels, the chloride content be determined and its Oxygen Consumed value (0.226 × wt. Cl^-) subtracted from the sample O.C.

4. Modifications and other oxidation procedures:

Several adjustments have been mentioned which extend the two methods to a variety of sample sizes and types. If the characteristics of the analyses described here are to apply to a modified procedure, three considerations are of particular importance:

a. An acid:water ratio of not less 2:1 by volume must be employed.

b. The stronger the dichromate concentration (up to about 2 *N*), the more effective will be the oxidation. Dichromate solutions less than 0.05 *N* should not be used.

c. The degree to which any sample is oxidized depends on the time-temperature relation of the reaction.

There are other dichromate oxidation procedures which may be useful. Those employing higher reaction temperatures can give a more rapid analysis. One of the simplest is Tiurin's (1931), which utilizes a lower acid:water ratio (about 1:1) in a 5-minute, direct-boiling reaction. A rapid procedure of this type may be desired in certain instances, but many of the characteristics that have been described here will not apply.

DETERMINATION OF ORGANIC ELEMENTS

Oxidative decomposition of organic compounds by acid dichromate in the methods just described differs only in minor respects from the digestions or combustions employed in common ultimate analyses. The end products are the same; Kjehldahl digestion producing NH_3 from organic nitrogen, and dry combustion achieving the transformation of organic carbon to CO_2. These inorganic forms of the two most important organic elements lend themselves to a variety of existing quantitative analyses. The problem posed was to find simple, direct techniques which could be incorporated into the dichromate oxidation scheme. Separation of CO_2 and NH_3 from the reaction mixture by diffusion and estimation by titration, as described below, appear to be the most suitable ones. These direct analyses can be complemented with a mathematical determination of hydrogen and oxygen in order to characterize all of the major elements in organic matter. The necessary calculations will be considered in part V.

Carbon analysis

Carbon dioxide is freely evolved from the acid oxidation mixture as the reaction progresses. There are many published methods treating the absorption and measurement of CO_2, and one of the simplest is that described by Baker et al. (1954). In essence, it is a nonquantitative wet oxidation in which a sealable jar serves as a combined combustion-diffusion vessel. Evolved CO_2 diffuses into an internally contained hydroxide solution and is measured after the reaction subsides by double titration with HCl. This procedure has been modified by Katz et al. (1954) for greater precision, and by Chen and Lauer (1957) for use with water-soluble materials. The following is adapted, in part, from the above papers. Since this determination becomes part of the quantitative oxidation procedure, its analytical range is limited by the size of the sample which can be used in a given oxidation. The oxidation procedure of the semimicro method is employed, so that the corresponding range of carbon detection is 5 to 20 milligrams. Microdetermination of carbon is possible by an appropriate adjustment of the absorbent concentration.

1. Apparatus and reagents:

All reagents and glassware listed for the semimicro method are required with the exception of the oxidation flasks. Below are the additional items needed for carbon absorption and titration.

a. Reaction-diffusion vessels: Erlenmeyer flasks, 125 to 250 ml., having single-hole rubber stoppers fitted with short lengths of rubber tubing and clamps. Alternatively, 250 ml. vacuum flasks with solid stoppers and clamped outlets may be used. Coat all stoppers very lightly with silicone grease.

b. Sample holders: 5-ml. beakers or similar flat-bottom glass cups which can be inserted into the reaction vessels.

c. CO_2 absorption tubes: Shortened test tubes or long vials, about 20 mm. in diameter, which fit up to (but not into) the neck of the reaction vessel when inserted.

d. Titration flasks: 125-ml. Erlenmeyer flasks with stoppers.

e. CO_2-free sodium hydroxide, approximately 2 *N*: prepare as needed from saturated stock (about 55 g. NaOH/100 ml. distilled water) by diluting about 16 ml. clear supernatant to 100 ml. with CO_2-free water.

g. Barium chloride, approximately 2 *N*: Dissolve 25 g. $BaCl_2{\cdot}2H_2O$ in 100 ml. CO_2-free water and store in a stoppered flask. If a precipitate forms, use only the clear supernatant solution.

h. Standard hydrochloric acid, 0.2 *N*: Dilute 19 ml. concentrated HCl to 1 liter with distilled water. Standardize by titrating against primary standard grade Na_2CO_3 (directions can be found in most general analytical texts).

i. pH indicators: Phenolphthalein—dissolve 1 g. in 70- to 90-percent ethanol; brom cresol green—thoroughly mix 0.1 g. brom cresol green with 14.3 ml. 0.01 *N* NaOH in a mortar (or in a beaker using a large stirring rod) and dilute to 100 ml. with distilled water.

2. Oxidation-diffusion procedure:

The semimicro method of oxidation is employed, including at least one reagent blank. However, all of the reactants cannot be combined before the reaction flasks are sealed. Therefore, the dichromate and acid are added directly into the flasks and the samples are placed in the sample cups which are inserted upright into the flasks. The following steps refer to each sample in a series.

a. If the sample contains inorganic carbon (carbonates or bicarbonates), moisten it with dilute sulfuric acid (5 percent) and allow several minutes for the CO_2 to diffuse out. CO_2 removal may be hastened by placing the acidified sample under vacuum. Samples containing large amounts of inorganic carbon require special pretreatment (Shaw, 1959).

b. With the reactants in place, insert an absorption tube containing 2 or 3 ml. of 2 *N* NaOH and stopper the flask (the hydroxide can be measured in the 10-ml. graduated cylinder). Draw a vacuum on the flask, and close the access clamp. Swirl or tilt the flask to overturn the sample cup, and continue swirling for about 30 seconds. If the absorption tube is of the proper length, there will be little danger of spilling the hydroxide.

c. Place the flask in a boiling water bath, following the oxidation procedure. The hydroxide may become desiccated toward the end of the reaction period, but apparently this does not affect CO_2 absorption.

d. Remove the flask from the bath, release the vacuum, and remove the absorption tube. The reaction solution adhering to the lower outer surface of the tube must be rinsed back into the flask. Stopper the tube if immediate analysis is inconvenient. Analyze the contents of the flask as in the oxidative method.

3. Carbon determination:

a. Rinse the contents of the absorption tube into a titration flask and dilute with CO_2-free water to about 20 ml.

b. Add 2 or 3 ml. of 2 *N* barium chloride (measured in the 10-ml. graduated cylinder) and swirl. A barium carbonate precipitate forms in proportion to the amount of CO_2 absorbed.

c. Add 1 drop of phenolphthalein and titrate with HCl until the color disappears. This acid volume is not recorded but the endpoint must be observed carefully.

d. Note burette reading, add 3 or 4 drops brom cresol green, and resume titrating to the yellow endpoint. The precipitate disappears as titration proceeds, and near the endpoint, color changes occur prematurely because of the free CO_2 in solution. When this occurs, expel the carbon dioxide by heating the flask to boiling. Cool it rapidly under running water, and resume titrating to the endpoint. Note the volume of acid used. The reagent blank will require a large volume of HCl to reach the phenolphthalein endpoint, but only a few drops in the final titration if reagents were properly prepared and contamination minimized.

4. Calculation of sample carbon content:

Mg. carbon = (sample titer − reagent blank titer) $\times$ *N* HCl $\times$ 6.

5. Example:

Two series of analyses of carbon in ammonium tartrate are given in table 5 with nitrogen determinations which will be described in the following section. Five ml. of 1 *N* dichromate and 10 ml. of acid were employed in oxidation. In one series, the tartrate was weighed and added in a dry state; in the other, dichromate was evaporated in the reaction flasks, and the tartrate was added as a solution of known concentration (5 ml. at 9.632 g/L.). The weights of carbon found have been converted to percentages to enable comparison of dissimilar samples. These analyses indicate an accuracy of 99 percent with a precision within ±2 percent of the mean value. Other easily oxidized compounds (e.g., oxalate, lactose, cellulose) have given similar results, but the method has not yet been used with resistant purified compounds. Limnological samples that have been analyzed by this method with only slightly lower precision are presented in part VI.

6. Comments:

The transformations of carbon in this procedure are summarized in the general reaction scheme:

organic carbon $+ O_2$——(oxidation)$\rightarrow CO_2$;

$CO_2 + 2NaOH$——(absorption) $\rightarrow Na_2CO_3$;

TABLE 5.—*Analyses of carbon and nitrogen in ammonium tartrate*

Element analyzed and sample weight (mg.)	Oxygen Equivalent [1]	Carbon		Nitrogen (duplicate series)			
		Milligrams found	Percent of sample	Milligrams found		Percent of sample	
				A	B	A	B
Carbon:							
51.7 mg.	0.429	13.50	26.2				
40.9 mg.	.433	10.49	25.7				
45.7 mg.	.434	11.72	25.6				
Mean	.432		25.8				
Nitrogen:							
40.9 mg.	.440			6.00	6.00	14.7	14.7
43.5 mg.	.427			6.51	6.50	15.0	14.9
41.6 mg.	.434			6.00	6.26	14.4	15.0
Mean	.434					14.8	
Carbon and nitrogen:							
48.16 mg.	.435	12.34	25.6	7.03	7.32	14.6	15.2
48.16 mg.	.435	12.73	26.4	7.70	7.35	16.0	15.2
48.16 mg.	.435	12.37	25.7	7.35	7.52	15.2	15.6
Mean	.435		25.9			15.3	
Mean percent of sample			25.9			15.1	
Mean percent of theory [2]			99.0			98.8	
Average deviation percent			±1.2			±2.3	
Coefficient of variation percent			±1.3			±3.0	

[1] Milligrams oxygen per milligram sample.
[2] Theoretical values are given in table 10.

$Na_2CO_3 + BaCl_2$ ——(precipitation) $\rightarrow BaCO_3$;
$BaCO_3 + HCl$ ——(titration) $\rightarrow CO_2$.

Carbon dioxide can be titrated as sodium carbonate, but precipitation with barium offers two benefits: it permits a sharp phenolphthalein endpoint (at pH 8.0) and it retains the carbon in a divalent state (equivalent weight = 6), thereby requiring twice the acid volume needed to titrate carbon as HCO_3^{-1}.

Two interferences probably occur to some extent in this procedure when complex organic matter is analyzed. Sample chloride, oxidizing to Cl_2 is expected to diffuse into the absorption tube and react to form chloride and hypochlorite. The presence of the latter has been manifest on occasion by the fading of phenolphthalein color (oxidation). A more important interference may be caused by the absorption of acetic acid (see part III), most of which would be titrated at the brom cresol green endpoint. This titration would account for only one-fourth of the carbon present as acetate. Although the occurrence of acetate is known only from indirect evidence, the circumstance, if true, could prove advantageous. The presence of hypochlorite might be used to advantage also. These two compounds (acetate and oxidized chloride) represent the major constant error in oxidative efficiency. An analysis of them in the absorption solution, which seems quite possible, would permit an insight into the composition of the sample and an accurate correction for the oxidation results.

Carbon dioxide diffuses rapidly from the reaction solution into the absorption tube. It is formed, however, at a definite rate which depends mainly on the stability of the organic matter; therefore, when natural organic matter, lipid, or proteinaceous material is oxidized, the flasks should be left unopened for about an hour after they are removed from the water bath. Another factor to consider is the water of the absorbing solution, which diffuses slowly into the reaction mixture because of the desiccating property of the acid and thereby lowers the acid concentration. Since this may reduce oxidative efficiency, a maximum hydroxide volume of 3 ml. has been suggested. A precaution which can be exercised is to begin the oxidation with a slightly higher acid:water ratio (e.g., 2.2:1). Evacuation of the reaction flasks is not an essential feature of this procedure, but merely hastens the diffusion process. Good carbon recoveries have been obtained by using unevacuated, 125-ml., screwcap Erlenmeyer flasks, allowing them to remain sealed for a few hours after removal from the water bath.

This diffusion analysis apparently can be applied readily to samples of the size employed in the micro method of oxidation. The method of Katz et al. (1954) concerns samples containing about 1 to 5 mg. carbon. Kay (1954) described a related technique in which carbon is estimated in 10-ml. samples of sea water (about 0.03 mg. C) with a precision of ±3 percent. Many modifications of this method are possible, and the interested reader is referred to Conway (1958) for a background on diffusion principles and applications, and to Kolthoff and Sandell (1952) for considerations of reagents, indicators, and reactions.

Nitrogen analysis

It has been noted that organic nitrogen appears as an ammonium salt in the dichromate oxidation reaction. A method was needed to estimate this nitrogen which would not prevent normal oxidative analysis. Ammonium nitrogen is stable in acid solution, but volatilizes as ammonia when the solution is made alkaline, as in a Kjehldahl distillation. Micro Kjehldahl distillations were, in fact, tried on small portions of the reaction mixture with varying success, but this procedure lacked the versatility desired for compatibility with the preceding analyses. Micro diffusion possibilities were investigated and a suitable procedure was developed on the general pattern of the Conway (1958) methods. The diffusion is accomplished with small aliquots of the reacted oxidation mixture in separate vessels. The ammonia is absorbed in a boric acid solution and titrated with HCl. The following procedure specifically concerns sample nitrogen contents representative of limnological material normally used in the semimicro reaction (0.5 mg. N or more).

1. Apparatus and reagents:

Except for the omission of silver sulfate in the acid (see "Comments," below), oxidation requires the same glassware and reagents as the semimicro method, with or without concommitant carbon analysis. They should be as ammonia-free as possible. The following items refer exclusively to the nitrogen analysis.

a. Diffusion dishes: Conway "units" with internal absorption wells are standard, but any covered dishes having optimal dimensions (Conway, 1958) may suffice. Stender dishes, 5 cm. in diameter with silicone grease in the lid grooves, were used here. Polyethylene vial caps, 25 mm. wide and 15 mm. deep, were satisfactory absorption cups.

b. Volumetric glassware: One 50-ml. volumetric flask for each sample and reagent blank in a series, a 1-ml. and a 3-ml. pipette, and 2-ml. Koch-type microburette are recommended. Larger burettes are usable with less concentrated acid.

c. Ammonia-free distilled water: Redistill from acidified solution, perfuse through an appropriate exchange resin, or shake with permutit (see A.P.H.A., 1955).

d. Mixed indicator, 0.1-percent alcoholic solution: Dissolve 33 mg. brom cresol green and 67 mg. methyl red in a few ml. 95-percent ethanol and dilute to 100 ml. with ethanol.

e. Boric acid absorbent, 1-percent solution with mixed indicator: Dissolve 10 g. H_3BO_3 in 700 ml. distilled water and 200 ml. 95-percent ethanol. Add 10 ml. indicator solution (above), mix, and make up to 1 liter. Adjust, if necessary, to a weak reddish tint (pH about 5) with a few drops of dilute NaOH.

f. Standard HCl, 0.01 *N*: prepare by diluting the standard acid described for carbon analysis with distilled water. The exact normality is calculated from the dilution ratio and determined normality of the stock HCl.

g. Potassium hydroxide, pellets: Fresh stock, low in carbonate.

2. Oxidation procedure:

The oxidation procedure of the semimicro method, with or without a carbon determination, is followed explicitly. It is assumed that the sample contains no reduced inorganic nitrogen (ammonium *N*). If such nitrogen is present, the sample should be treated with a small amount of dilute hydroxide and the liberated ammonia allowed to diffuse out before proceeding with the oxidation. After the oxidation is completed, the reacted mixture is rinsed into a 50-ml. volumetric flask with enough ammonia-free water to make a total diluted volume of about 49 ml. This solution is mixed well, allowed to cool, and made up to 50 ml. At least half of it (25 ml. or more) is used for the Oxygen Consumed determination. The remainder can be stored tightly stoppered if immediate nitrogen analysis is inconvenient.

3. Nitrogen diffusion and titration:

A precisely measured amount, from 1 to 5 ml., of the oxidized sample solution is required for each nitrogen determination. Any amount taken below 5 ml. must be diluted with ammonia-free water to this volume, which is optimum for the dish size and neutralization conditions specified here. The following operations refer to an "average" aliquot of 3 ml. Effective neutralization of this solution requires manipulative proficiency which is easily attained with a little experience. Neutralization must be complete in every respect and the only acid present in the diffusion dish after that time should be within the absorption cup. As in previous procedures, it is expedient to analyze a series of samples simultaneously, reagent blank included. A single member of such a series is described below.

a. Pipette a 3-ml. aliquot of the sample solution into a clean diffusion dish, add 2 ml. ammonia-free water (it can be measured in a small graduated cylinder), and swirl the dish gently to mix the contents.

b. Count out the number of KOH pellets needed to neutralize this solution, and include one additional pellet. The necessary amount of hydroxide can be estimated from the supposed acid content of the sample aliquot. In this case, it will be about 1.2 g. or 6 to 8 pellets. Assume that 7 pellets, including the extra one, are required. Add 3 of them to the dish while gently swirling it and continue to swirl until the pellets have dissolved. At no time should the pellets be exposed above the surface or allowed to effervesce, else a premature evolution of ammonia will occur. Add 2 more pellets and repeat the above operation. When these have dissolved, the solution should still be acid and light amber in color (cf., reaction of dichromate with hydroxide in part III). The pH can be tested by touching the tip of an indicator paper to the surface of the solution. Cover the dish lightly and set it aside.

c. Pipette 1 ml. of boric acid into the absorption cup. Precise measurement is unnecessary, but all members of the series should contain about the same amount. If the nitrogen content of the aliquot is more than 0.2 mg., use 1.5 ml. absorbent.

d. Remove the lid from the diffusion dish, place the absorption cup inside, and with the lid in readiness place the remaining 2 KOH pellets in the partly neutralized solution. Quickly press the lid into place, and tilt the dish with a precessing motion to dissolve the pellets (the sample solution should not, of course, contact the absorbing solution). The sample solution should now be alkaline. This fact will be evidenced by a white precipitate which forms during the final neutralization and by the color change of the chromium compounds (see part III). Sample solutions will usually turn light green, and the reagent blank, a light yellow. Another indication of proper pH change is the color change of the absorbent, which becomes green after a few minutes as a result of ammonia absorption. Heat evolved in the final neutralization may cause temporary internal pressure, making it advisable to clamp or weight the lid until the dish has cooled.

e. Set the dish aside for a few hours (preferably overnight) until ammonia diffusion is complete. An occasional rotation of the dish during this period will hasten diffusion.

f. Remove the absorption cup, and titrate its contents in situ to a red endpoint with standard HCl. Various but distinct shades of red are possible. If a reagent blank is titrated first (it should not require more than a drop or two of acid), the samples can be titrated to the same color with ease.

4. Calculation of organic nitrogen content:

The nitrogen content of the aliquot analyzed is given by the equation

$$\text{mg. N} = (\text{sample titer} - \text{reagent blank titer}) \times N\ \text{HCl} \times 14,$$

and the nitrogen content of the original sample will be

$$\text{sample N, mg.} = \frac{\text{mg. N in aliquot} \times 50}{\text{ml. aliquot}}.$$

5. Example:

Nitrogen recoveries from two ammonium tartrate oxidation series are reported in table 5, one of them done in conjunction with organic carbon determination. Duplicate aliquots were analyzed for each sample, and the nitrogen quantities found were converted to percentages of sample weight for comparative purposes. The accuracy of the method on this compound is 99 percent of the theoretical value, with a precision of 3 percent (coefficient of variation). The accuracy and precision with other pure compounds have not yet been tested, but favorable results were obtained with limnological samples, as described in part VI.

6. Comments:

Some advantages of this method are: it is adaptable to a large number of concurrent analyses; it allows normal oxidative analysis, even when replicate aliquots of the sample solution are taken; only one standard solution, one exact pipetting, and one titration are required for each determination; diffusion units are simple and may be fashioned from various items of laboratory glassware. The chief disadvantages are the time required for diffusion and the care needed to avoid contamination when working with low levels of ammonia N. The diffusion period, however, can be devoted to other activities, since the process requires no attention once the units have been prepared.

A black precipitate was observed to form in the neutralized solution when the oxidation included catalytic silver. Silver sulfate was omitted thereafter, since the effect of this precipitate on ammonia diffusion is unknown. The absence of silver is expected to lower the completeness of oxidation of resistant compounds by a few percentage points (see part III). This reduced efficiency primarily affects carbon recoveries (cf., pages 9 and 13). All organic N, imino N possibly excepted, should be converted to ammonium salts if the sample is thoroughly exposed to the oxidizing mixture.

Quite probably, the silver precipitate does not interfere with nitrogen analysis—a premise which will be tested in future work.

Both the precision and the accuracy of the method reside mainly in the diffusion process. The rate of ammonia transfer, from the sample solution into the absorbent, is controlled principally by its diffusion rate within the former solution. The surface area of this solution with respect to its depth becomes a limiting factor which is affected directly by the dimensions of the diffusion vessel. Diffusion rates will also vary with the neutralizing agent used, but are not dependent upon the final alkalinity attained (so long as ammonium is converted to ammonia). These factors are discussed at length by Conway (1958). For any given diffusion vessel, the smallest volume of sample solution which can cover the bottom area will be the most efficient. Strong acidity of this solution in the present procedure imposes limits on the volume employed and the type of neutralizing agent which can be used effectively.

Concluding remarks

These methods of organic carbon and nitrogen analysis have not been investigated as thoroughly as the oxidation technique. They conform well with the objectives of method simplicity and versatility which are desired, and appear to be useful limnologically in their present state of development. If the methods are employed in this capacity, it is recommended that reagent grade ammonium tartrate be included in occasional analyses as a standard substrate. Further testing is expected to result in improved procedures and in a better knowledge of the character and reliability of the results. Data given by these methods can be combined with oxidation values to provide additional organic characterizations which are described in the following text.

Part V. SIGNIFICANCE AND UTILIZATION OF ANALYTICAL DATA

Oxidation values obtained with the foregoing procedures are applicable to the estimation of organic mass, energy, and composition. The requisite conversion factors, however, must be derived from considerations of the relation of oxidation oxygen to organic matter. In the following treatment, emphasis is placed on the characteristics of organic matter from natural environments because data from laboratory cultures are biased by unnatural conditions. Several concepts require supporting information from an interpretation of natural organic matter according to prominent compounds and proximate groupings. This interpretation proves too voluminous for direct inclusion in the text, and has therefore been deferred to the appendix.

TRANSFORMATIONS OF OXYGEN CONSUMED VALUES

The transformation of an Oxygen Consumed datum into more cogent organic values requires numerical conversion factors which express the desired relations. Since organic matter in any limnological sample is a broad assemblage of various compounds, these factors must necessarily be averages which carry certain assumptive risks. An attempt has been made to minimize errors of assumption by an extensive consideration of the relevant characteristics of natural organic matter. Three conversions of the O.C. value described below (to total organic matter, organic carbon, and caloric content) are direct, 1-step transformations, and a fourth employs both O.C. and elemental composition data in estimating total organic matter from calculated proximate components.

Direct estimation of total organic matter

The factor which converts the Oxygen Consumed by a sample to organic weight is the theoretical Oxygen Equivalent of the organic matter it contains. For instance, the milligrams of oxygen reacting with any amount of a pure organic compound, when divided by its O.E. (mg. oxygen per mg. compound), will give the weight of the compound that was oxidized. To convert the O.C. values of natural samples to organic weights, it is necessary to assume a single O.E. that is universally representative. Some proposed conversion factors have been based on the O.E. of a single nonrepresentative compound, such as a hexose sugar (O.E. = 1.06). Vinberg (1954) has employed the relation 1 mg. oxygen = 0.75 mg. ashless plankton (or O.E. = 1.33) in the study of lake seston.

A universal factor may be determined empirically from the oxidative analyses of samples whose organic content is known with a reasonable degree of certainty. An example is given in table 6, which lists both the O.C. values (as weight ratios) and the experimental O.E.'s. of seven limnological substrates. The former values are included to show the variability which occurs among them as a result of different ash and residual moisture contents. The O.E.'s. fall into a rather narrow range (1.38 to 1.46) with a median of 1.42 mg. O/mg. organic matter. These few samples, however, do not provide an unequivocal basis for assuming a universal conversion factor. Prominent sample types missing are seston, sediments, and total dissolved organic matter. Many limnological materials are not available in sufficient quantity or are proportionately too low in organic content to permit this sort of interpretation. It is necessary, therefore, to use other methods to determine a representative O.E.

Another way in which the Oxygen Equivalent can be determined for a mixture of unknown organic compounds, such as a natural sample, was described in part III. The O.E. is calculated from a hypothetical molecule whose atomic ratio is derived from the ultimate composition of the mixture. A few relevant ultimate analyses found in limnological literature have been recalculated as indicated, and their data are presented in table 7. These O.E.'s are more variable than the ones above, ranging from 1.20 to 1.71 mg. O/mg. sample. The number and classes of samples also are too few to give a representative "average" value.

A final approach to the determination of a universal conversion factor (O.C. to organic matter) is made through an evaluation of proximate analyses selected from several literature sources. This method of assigning O.E.'s. to

TABLE 6.—*Experimental Oxygen Equivalents and related analytical factors for some natural organic substrates*

Factor	*Aphanizomenon*	*Batrachospermum*	*Cladophora*	*Daphnia*	*Gloeotrichia*	Humic extract	Neuston
Oxygen Consumed (air-dry basis)	1.22	1.09	1.12	1.04	1.21	0.67	1.18
Moisture + ash percent	8.9	26.8	28.1	25.1	9.8	51.1	13.2
Oxygen Equivalent [1]	1.41	1.38	1.44	1.46	1.41	1.44	1.43
Source [2]	A	B	B	A	A	C	D

[1] Calculated from Oxygen Consumed values by adjusting them to a moisture + ash-free basis and allowing for 5 percent incomplete oxidation.

[2] Sources of substrates: A are plankton organisms from Crowley Lake, Calif.; B are attached algae from Beaver Brook, Cortland County, N. Y.; C is a precipitate (by $CuSO_4$) from acid lake water taken in Adirondack State Park, N. Y.; D is a surface film (microorganisms) obtained from a pond in Cortland County, N. Y.

TABLE 7.—*Oxygen Equivalents of some limnological substrates calculated from their reported elemental compositions*

Substrate	Elemental atomic ratios				Valence change [1]	Oxygen Equivalent
	Carbon	Hydrogen	Oxygen	Nitrogen		
Copepods [2]	4.43	8.13	1.67	0.87	19.90	1.59
Peridineans [2]	4.02	7.39	2.12	0.75	16.98	1.36
Diatoms [2]	4.20	10.10	1.80	0.74	21.40	1.71
Humic precipitate [3]	4.10	4.46	2.74	0.17	14.97	1.20
Humolimnic acid [4]	4.53	5.59	2.44	0.09	18.56	1.48
Crenic acid [5]	4.14	8.22	2.48	0.16	19.34	1.55

[1] Valence change in oxidation per hypothetical molecule, molecular weight = 100.

[2] Brandt and Raben (1920). O.E. range for all diatom analyses reported was 1.60 to 2.01.

[3] Åberg and Rodhe (1942).

[4] Shapiro (1957). Acetate extract of lake water.

[5] Ponomareva and Ettinger (1953). Electrodialyzate of Neva River water.

samples is actually a 3-step process based on the premise that a group of chemically related compounds can be represented fairly accurately by a single O.E. The first two steps (i.e., the arrangement of limnologically important compounds into related proximate groups, and the characterization of each

TABLE 8.—*Oxygen Equivalents calculated for various limnological substrates from their reported proximate compositions*

Reference and substrate	Sample code [1]	Number of analyses	Percent			Oxygen Equivalent [2]
			Crude protein	Lipid	Crude carbohydrate	
Birge and Juday (1934):						
Raw lake water, carbon per liter:						
1–1.9 mg.	D	(data from 529 lakes)	26.4	2.4	71.2	1.46
5–5.9 mg.	D	do	21.4	1.6	77.0	1.44
10–10.9 mg.	D	do	16.9	0.7	82.4	1.43
15–15.9 mg.	D	do	14.5	0.5	85.0	1.42
20–25.8 mg.	D	do	12.3	0.3	87.4	1.42
Weighted mean			17.2	0.9	81.9	1.43
Seston	Sn	529 lakes	37	4	59	1.39
Organic sediments	St	66	26.5	2.6	70.9	1.46
Dissolved organic matter:						
Diamond Lake, 3.26 mg. per liter	D	unknown	28.5	3.3	68.2	1.48
Helmet Lake, 55.3 mg. per liter	D	unknown	8.1	0.6	91.3	1.42
Birge and Juday (1922):						
Net plankton:						
Mendota Lake	N	84	51.2	12.2	35.7	1.55
Monona Lake	N	21	62.8	7.0	30.2	1.50
Waubesa Lake	N	16	49.5	4.0	45.5	1.40
Nannoplankton:						
Mendota Lake	Sn	87	42.8	6.6	50.6	1.43
Monona Lake	Sn	21	55.8	4.8	39.4	1.44
Waubesa Lake	Sn	16	46.8	5.8	47.4	1.43
Organism groups:						
Cyanophyta (5 genera)	P	10	57.2	3.2	39.6	1.45
Volvox	P	1	50.8	5.9	43.3	1.47
Diatoms	U	1	37.8	22.5	39.7	1.65
Copepoda (2 genera)	I	4	63.2	16.5	20.3	1.62
Cladocera (3 genera)	I	14	59.3	11.8	28.9	1.54
Aquatic insecta (5 orders)	I	7	62.0	12.0	26.0	1.55
Geng (1925): Aquatic invertebrates:						
Insecta (5 orders)	I	7	63.0	11.4	25.6	1.54
Crustacea (3 orders)	I	5	71.1	8.1	20.8	1.51
Mollusca (3 genera)	I	4	54.3	7.4	38.3	1.48
Hsiao (1949):						
Zooplankton	I	3	65.9	19.3	14.8	1.69
Phytoplankton	P	7	45.0	7.3	47.7	1.40
Krogh and Lange (1932): Lake Furesø samples:						
Plankton	N	unknown	53.2	9.9	36.9	1.52
Colloidal matter	U	12	41.5	11.7	46.8	1.60
Dissolved matter	D	15	37.5		62.5	1.44
Krogh and Berg (1931): Schlosssee plankton	N	18	53.6	10.7	35.7	1.53
Matida and Sakurai (1953): River periphyton	P	2	21	9	70	1.40

[1] Samples are coded by the type of organic matter they contain: D = dissolved organic matter; I= aquatic invertebrates; N = net plankton (probably mainly zooplankton); P = phytoplankton (and other plant cellular material); Sn = seston; St = organic sediments; U = unclassified.

[2] Factors assumed for proximate groups in calculating total Oxygen Equivalent: O.E.$_P$ = 1.5; O.E.$_L$ = 2.7; O.E.$_C$ = 1.2 (except D and St samples where O.E.$_C$ = 1.4).

group by its representative O.E.) have been accomplished in the appendix. The last step is indicated in table 8, which lists various proximate analyses that have been recalculated to ash-free unit values where necessary. A sample O.E. is found by summing the products of the proximate component proportions times the respective O.E. constants.

This procedure is less reliable for a single sample than the ones described previously because it assumes true representation by the O.E. constants, and accuracy of the proximate analyses. The resulting error is expected to decrease as individual analyses are combined, and therefore greater emphasis should be placed on the O.E.'s. calculated from the data which are averages of many analyses. The abundance of data in table 8 permits considerations of sample types, but such considerations can be made only in general terms because of many minor confounding differences among the various analyses. The following is an interpretation of the characteristic O.E.'s. of the different sample types noted in table 8 (sample types are listed in the approximate order of decreasing limnological importance):

Sample type	*Table code*	*O.E. range*	*Representative O.E.*
Dissolved matter	D	1.42–1.47	1.44
Seston	Sn	1.39–1.53	1.40
Organic sediment	St		1.46
Phytoplankton	P	1.40–1.47	1.44
Aquatic invertebrates	I	1.48–1.69	1.54
Net plankton	N	1.40–1.55	1.52

It is not possible to classify the datum on colloidal organic matter into this scheme. The single diatom datum was omitted because diatoms are known to contain large amounts of lipid under certain conditions and in this respect are similar to plankton crustaceans (see table 7). The direct relation between lipid content and O.E. is strongly indicated in table 8.

The three methods of determining sample O.E.'s. give results that are in general agreement. The most representative values appear to be in the range 1.4 — 1.5 mg. O/mg. sample organic matter, although high-lipid substrates have O.E.'s. that are definitely higher. The types of samples most concerned with the conversion factor being determined are dissolved organic matter, seston, and sedimented matter. The O.E. characteristic of these samples, 1.43, is selected as a "universal" factor for converting O.C. values to organic weight. This may be accomplished by division, but it is more convenient to multiply an O.C. by the reciprocal, 1/1.43, which may be rounded to 0.7. The relative error involved in using this factor for comparable samples will be small. The approximate absolute error can be shown by considering "true" O.E.'s. at the extremes of the normal range. Assume that two different samples have actual O.E.'s. of 1.3 and 1.7. If their O.C. values (observed) are converted to organic weight by the suggested factor (1.43) the estimate will be 9 percent too low in the first case and 19 percent too high in the second. Generally, however, calculated organic weight is expected to be within ±10 percent of the true value, assuming perfect analytical accuracy.

Estimation of organic carbon

Although the analytical methods described here include the direct determination of organic carbon, it may be desirable, instead, to estimate the sample organic carbon content from the analytical Oxygen Consumed. This conversion is actually less subject to assumptive error than the conversion to organic matter because the O.C. varies with carbon content more closely than it does with organic mass.[2] The factor that transforms O.C. to carbon is the Oxygen Equivalent of carbon, which is the O.E. of a compound divided by its fractional carbon content. In part III, the carbon O.E. is indicated as the effective valence change of carbon in a molecular oxidation, times 8 (to get oxygen weight), divided by the weight of carbon in the molecule. The O.E. factor needed for the conversion of any observed O.C. to organic carbon, as for the conversion to organic matter, is a single value which is representative of the effective valence state of organic carbon in limnological samples.

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[2] Referring to the values obtained in the appendix, 1 gram of oxygen will oxidize 0.83 gram of crude carbohydrate containing 0.38 gram of carbon, but will oxidize only 0.37 gram of lipid containing 0.28 gram of carbon.

The conversion to organic carbon is frequently given in the literature on the basis of a carbon valence change of 4 electrons (see part III) i.e., that elemental carbon or true carbohydrate is being oxidized. The transformation described by El Wakeel and Riley (1957) serves as an example (it was adapted from Allison, 1935):

$$\text{ml. } 0.2N\ \mathrm{Fe}^{+2} \times 0.6 \times 1.15 = \text{mg. carbon.}$$

The first term of the equation is the net titer, each milliliter being the equivalent of 1.6 mg. of oxygen. The second term indicates that 0.6 mg. of carbon are equivalent to the first term (or that 32 mg. of oxygen are equivalent to 1 mg. atomic weight of carbon). The third term, 1.15, is stated to be a factor which corrects the calculated carbon to the true weight as determined by dry combustion analysis. It is, in fact, a factor which corrects partly for incomplete oxidation and partly for the inappropriate value of the second term (according to the valence interpretation discussed here in part III).

An appropriate conversion factor can be found by treating natural organic matter in a manner similar to that employed above in finding an O.E. representative of limnological substrates. This approach is considered unnecessarily meticulous because the carbon can be determined directly, if necessary. It is obtainable more easily by considering the average amount of carbon in natural organic matter and then properly adjusting the "universal" O.E. to a carbon O.E. Waksman and Hutchings (1935) discussed the carbon contents of various natural organic materials and indicated a carbon range of 50 percent to 58 percent in the organic matter of plant residues, humuses, peats, and composts. The data of Birge and Juday (1934) show carbon percentages of 46 to 48 in dissolved and sestonic organic matter. Of 17 plankton analyses presented by Brandt and Raben (1920), 15 place carbon between 47 percent and 53 percent of sample organic weight. Skopintsev (1948) stated that the carbon content of organic matter in river waters near Moscow is usually near 50 percent. The consensus seems to be that natural organic matter is very nearly 50 percent carbon. The representative carbon O.E. is therefore 1.43 × 2 (or 2.86). Its reciprocal (1/2.86 or 0.35) is a multiplication factor for converting O.C. to organic carbon.

The error of assumption in this conversion can be roughly described by assuming a certain range for the actual carbon content of the sample organic matter. If it is allowed that most limnological organic matter contains 46 to 54 percent carbon, the calculated carbon content for any sample will be within ±8 percent of the true value if the O.C. and assumed O.E. are accurate. It is possible to describe this error relative to the error in calculating organic content by a consideration of the theoretical O.C. and carbon content of a hypothetical sample, and values calculated therefrom. If this is done, it will be apparent that the calculation error for carbon is only half that of the organic matter error. In most circumstances where the oxidation is accurate, the calculated carbon content of a sample should be within ±5 percent of the true value.

Estimation of proximate composition

It is possible to calculate the proximate composition of sample organic matter in terms of weights from the analytical values for O.C. carbon, and nitrogen. The method, however, involves several independent assumptions and is less reliable than a different method described in the following section. For this reason, only a brief outline of the calculation will be given here.

The foregoing conversions and the considerations in the appendix point out the relations that exist between proximate groups, their carbon content, and their assumed Oxygen Equivalents. These relations can be combined in two parallel equations which are solved by inserting three analytical values. The values for O.C. and weight of carbon are employed directly, while the organic nitrogen is converted first to crude protein. The latter derived value is used, in turn, to calculate the weight of protein carbon, which is the third value needed. The equations are:

$$(1) \qquad C_C + \qquad C_P + \qquad C_L = C_T$$

$$(2) \quad 2.66\ C_C + 2.89\ C_P + 3.86\ C_L = \text{O.C.},$$

where C_C, C_P, and C_L are the weights of carbon in crude carbohydrate, crude protein, and lipid, respectively; C_T is the total organic carbon in the sample; O.C. is the sample Oxygen Consumed.

The numerical constants 2.66, 2.89, and 3.86 are carbon O.E.'s for the respective proximate groups (K = group O.E. × 100/percent carbon) and serve to convert carbon weights to oxygen weights. Analytical values for C_T and O.C., in the same weight units, are inserted directly. C_P is calculated for the sample:

$$\text{Weight protein carbon} = \frac{\text{weight organic N} \times 6.25 \times 100}{52}$$

and is inserted in both equations. Equation 1 is then multiplied through by 2.66 and subtracted from equation 2, giving a final equation containing one unknown, C_L, which is easily calculated. C_C is the difference, $C_T - (C_P + C_L)$. The weight of the proximate groups is obtained from their assumed carbon percentages:

$$\text{Weight crude carbohydrate} = C_C \times 100/45,$$

$$\text{Weight lipid} = C_L \times 100/70.$$

Crude Protein weight has already been determined (weight organic N × 6.25). Finally, the weight of the proximate groups can be summed to give an estimate of the total weight of organic matter in the sample.

Estimation of organic energy

The potential energy of organic matter in biosystems is related more to the state of reduction than to the organic mass, and is nearly always measured as heat (calories) by combustion calorimetry. These general associations have been discussed by West (1956), who also pointed out that the total energy given off by a substrate in oxidation is independent of the pathway as long as the end products are identical. While the temperature changes observed in combustion calorimetry provide a standard method of measuring organic energy, the relation between this energy and Oxygen Consumed is close enough to permit an accurate caloric estimate by quantitative oxidation. The importance of this relation was stressed by Spoehr and Milner (1949). Oxidative analyses have been proposed for organic energy characterization by Ivlev (1934) and Krogh (1930), and applied in the studies of Krogh and Berg (1931), Krogh and Lange (1932), and Vinberg et al. (1934). The transformation of an Oxygen Consumed datum to caloric value involves less assumptive risk than the previously described conversions, and requires a conversion factor representative of limnological organic matter.

The energy liberated in organic oxidation was described by Kharasch (1929) as the result of a rearrangement of interatomic bonds from higher to lower energy states. Different types of bonds represent different energy levels. Kharasch has taken the bond energy of methane oxidation in terms of valence change as a base value (26.05 kilocalories per electron mole) to demonstrate a method for calculating heats of combustion for pure compounds with an accuracy of about 1 percent. The method involves multiplying electron moles required for oxidation (valence change) by 26.05 and adjusting the product to a final value with certain energy factors attributable to specific configurations. Earlier, Thornton (1917) had deduced a combustion caloric value of 53 kilocalories per gram atom of oxygen by comparing observed heats of combustion with the amounts of oxygen required. Since 1 gram atom of oxygen equals 2 electron moles, the two conversion bases are in close agreement. Kharasch's adjustments are mostly addition factors, so the relation 26.5 kilocalories per electron mole (or 3.31 kcal./g. oxygen) can be taken as representative.

The conversion factors of both Kharasch and Thornton are based primarily on hydrocarbons which have less energy per unit weight of Oxygen Consumed than many biologically eminent compounds. The differences are not large, but a more representative factor is obtained by a consideration of the caloric and oxidation values of important limnological compounds. This has been done in the appendix by dividing the available standard thermodynamic heats of combustion (as kcal./g.) by the theoretical O.E.'s. of the respective substrates. Rounded values assumed for coefficients of the proximate groups from these data (as kcal./g. oxygen) are: Crude carbohydrate, 3.5; crude protein, 3.3; lipid, 3.3. The single representative value selected is 3.4 (or 27.2 kcal./ electron mole) which agrees closely with the factor 3.38 suggested by Ivlev (1934) and Vinberg et al. (1934). More often gram calories are calculated because the analytical oxygen datum is expressed in milligrams. The equation is: Sample gcal. = O.C. in mg. × 3.4.

The total calories found in this manner for nitrogen-containing samples do not compare directly with bomb calorimeter results because the latter assumes the oxidation of organic nitrogen to N_2. In most cases, biologically available energy does not concern nitrogen oxidation calories, and the decision as to which expression is most appropriate rests with the investigator. Calorimeter and oxidation data for nitrogenous materials can be adjusted to mutual agreement by appropriate addition or subtraction of nitrogen calories obtained from the relation N kcal. = g. organic N × 5.5. This relation is not exact because organic nitrogen bonds exist at various energy levels that cannot be evaluated easily.

Ivlev (1934) has calculated oxycalorie coefficients for 16 invertebrates (analyses reported from Geng, 1925, and Brandt and Raben, 1920) and found them to vary between 3.33 and 3.49. His own data indicated an accuracy of oxidation calorie determinations within ±5 percent of bomb calorimetry values. The error associated with the assumed conversion factor is not very large. If the true oxycalorie coefficients for natural samples vary between the extremes indicated for cellulose (3.53) and palmitic acid (3.26), the calculated calories will be within ±4 percent of the actual calories.

Since the Oxygen Consumed value of a sample more accurately represents organic energy, its conversion to calories is preferred to the other transformations described. The ratio of calories to carbon weight (determined, not calculated) can be useful in characterizing the organic energy level of a sample whose content of total organic matter is unknown. Fat, for instance, is distinguished by its high energy content per unit weight (about 9.4 gcal./mg.), which can be expressed in terms of its carbon content: 12.6 gcal./mg. carbon. Different compounds and also different samples have characteristic calorie/carbon ratios ranging up to 17.6 (methane).

UTILIZATION OF OXYGEN EQUIVALENT VALUES

An oxidative analysis is valuable even if the organic content of a sample is known. In this instance, the reaction serves as the first step in carbon and nitrogen analysis, and the quantity of oxygen which reacts provides a measure of the energy content of the sample. No less important is the experimental Oxygen Equivalent, which is used with the organic weight and the measured quantities of carbon and nitrogen to determine the proximate and ultimate compositions of the sample.

Determination of proximate composition

The nature of proximate groups with respect to limnological matter is treated in the appendix. In theory, these groups represent pure or closely related compounds. In analysis, however, total organic matter is merely partitioned by the methods employed into three groups which then contain some compounds that can be quite unrelated. Obviously, no two radically different methods will give identical results, nor can either be adjudged as more or less accurate so long as their results represent the defined or implied categories. The following method attempts to evaluate proximate groups analogous to the most common determinations: Crude protein is, by definition, organic nitrogen times 6.25; lipid conforms to organic extraction analysis, with a divergence described in the example; crude carbohydrate is the residual which brings the total to sample unity.

At least two oxidative methods of proximate group analysis have been described for limnological samples. Krogh (1930) used a dry combustion technique with organic carbon, nitrogen, and Oxygen Consumed measured by gasometry. These values were interpreted by respiratory quotients to give proximate groups. Spoehr and Milner (1949) derived proximate groups from ultimate components, also analyzed by dry combustion. Their calculations are similar to those given below, but use the "R-value" noted in part III. Their method has been used to estimate the proximate composition of cultured algae (Spoehr and Milner, 1949; Milner, 1948; Ketchum and Redfield, 1949).

The calculation of sample proximate composition requires two analytical values (in addition to the weight of organic matter), the O.E. and the organic nitrogen content, and three constants (proximate group O.E.'s.) taken from the appendix. Proximate percentages can then be derived from the parallel equations:

$$(1) \qquad \%CC + \%CP + \%L = 100$$

$$(2) \qquad 1.2\ \%CC + 1.5\ \%CP + 2.7\ \%L = \text{O.E.} \times 100,$$

where CC, CP, and L are crude carbohydrate, crude protein, and lipid, respectively. The quantity of nitrogen determined is converted to its percentage of organic matter and multiplied by 6.25 to obtain %CP. The latter is inserted into both equations. Equation (1) is then multiplied through by 1.2 and subtracted from equation (2), which cancels out the first term. The result is factored and rearranged:

$$(3)\ 1.5\ \%L = 100(\text{O.E.} - 1.2) - 0.3\ \%CP,$$

which is solved to obtain the lipid percentage. The crude carbohydrate percentage is then found by difference: $\%CC = 100 - (\%CP + \%L)$.

These determinations are frequently carried out on a large number of samples and the calculations may then become tedious. This condition can be averted by using a nomogram as shown in figure 4, which was prepared from equation 3 according to the method described by Levens (1948).

This oxidative determination of proximate composition is demonstrated in table 9 and compared with the results of "standard" analyses (crude protein from micro Kjehldahl nitrogen and lipid by ether extraction). The samples, trout fingerling and developing trout eggs, were the subjects of a physiological study reported by Phillips et al. (1959), and the "standard" compositions were taken from the original analytical data of that study. "Standard" protein values were used in the oxidative determinations because crude protein is synonymous in the two methods. Since both methods yield crude carbohydrate by difference, the comparison is more directly that of the lipid values.

A good agreement exists between the two methods for the percentages of lipid and crude carbohydrate found in trout fingerling. However, a striking difference is manifest in these values for trout eggs; the percentage lipid determined by oxidation is 2 to 3 times higher than by ether extraction. This anomaly is attributable to the nature of trout egg lipid, which Smith (1952) described as being distributed about equally between triglycerides (nonpolar) and phospha-

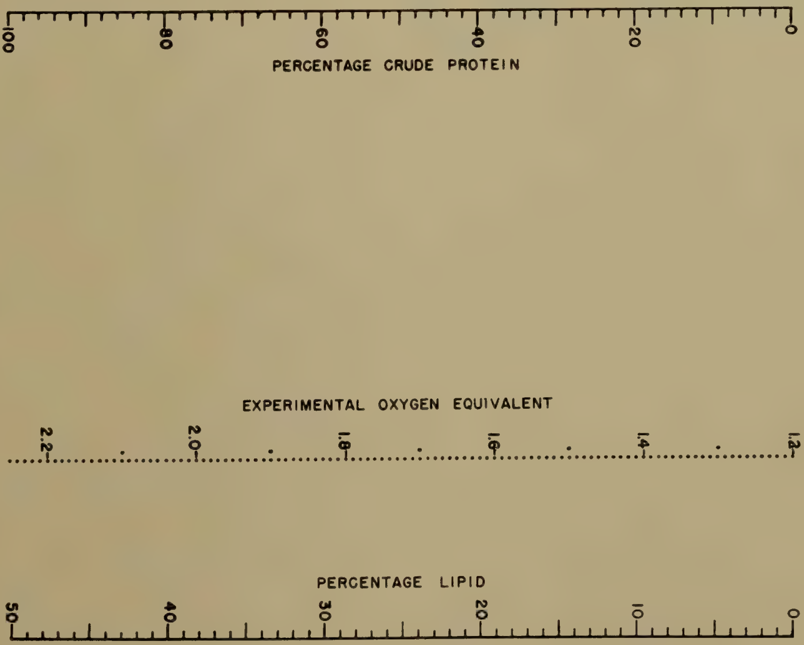

FIGURE 4.—Nomogram for the determination of lipid percentages from crude protein percentages and experimental Oxygen Equivalents. Lipid percentage is obtained by extrapolating a line connecting the two points representing the empirical values.

TABLE 9.—*Comparison of proximate compositions of trout samples determined by oxidative and "standard" analytical methods*

Sample Substrate [1]	Oxygen Equivalent	Percentage compositions [2]				
		"Standard" analysis			Oxidative analyses	
		Crude protein	Lipid	Crude carbohydrate	Lipid	Crude carbohydrate
Fingerling:						
Lot 1	1.69	60	22	18	21	19
Lot 2	1.80	48	33	19	30	22
Lot 3	1.64	59	19	22	17	24
Lot 4	1.81	47	30	23	32	21
Eggs:						
Lot 21	1.75	71	9	20	23	6
Lot 22	1.75	66	8	26	24	10
Lot 44	1.75	64	13	23	24	12
Lot 17	1.76	67	11	22	24	9

[1] Values for trout fingerling are means of 4 or more determinations; values for trout eggs are means of 2 or more determinations.

[2] "Standard" analysis by micro Kjehldahl and continuous ether extraction (Phillips et al., 1959); quantitative oxidation by the semimicro method. The same protein values apply to both analyses.

tides (polar). Smith cited data from several sources which indicate that total trout egg lipid is usually more than 20 percent of the total dry weight. He also suggested that relatively nonpolar solvents (ether, carbon tetrachloride) effectively extract only triglyceride fat. The oxidative determination is not founded on solubilities and will therefore give lipid values comprising all compounds distinguished by their strongly reduced state, or high energy content.

The equations and nomogram given here are based on one set of proximate group O.E.'s. which are assumed to be generally representative. Certain samples whose molecular composition differs strongly may be better characterized by substituting more appropriate constants (see appendix).

Determination of ultimate composition

The percentages of any two organic elements in a sample can be calculated from its empirical O.E. by parallel equations if the content of the remaining organic elements is known. The procedure has been described by Kuhn and L'Orsa (1931) and by Williams (1937). The four major organic elements, C, H, O, and N are of greatest concern, and the method will be explained for them. Carbon and nitrogen are determined analytically as previously described, and their percentages in the sample are used with the sample O.E. to calculate the hydrogen and oxygen percentages. The equations and procedure are similar to those used in proximate group determination:

$$(1)\quad \%C + \%H + \%N + \%O = 100$$

$$(2)\quad 2.7\ \%C + 7.9\ \%H - 0.7\ \%N - 1.0\ \%O = \text{O.E.} \times 100.$$

Letters designate the elements concerned. The numerical coefficients are true constants which have been calculated from the relation

$$\text{constant} = \frac{\text{initial valence} \times \text{equivalent weight oxygen}}{\text{atomic weight}}$$

and carry the sign of their valence number (C^{+4}, H^{+1}, N^{-3}, O^{-2}). The O.E. is the sample Oxygen Equivalent.

The hydrogen percentage is calculated by inserting the three empirical values (%C, %N, O.E.) into both equations 1 and 2. The equations are then added to cancel out the oxygen terms, and the result is factored and rearranged:

$$(3)\quad 8.9\ \%H = 100\ (\text{O.E.} + 1) + 0.7\ \%N - 3.7\ \%C.$$

TABLE 10.—*The ultimate composition of ammonium tartrate as determined by oxidative analysis*

Element	Percentage composition			Related data
	Experimental		Theoretical	
	Analyzed	Calculated		
Carbon	25.9		26.1	Formula: $C_4H_{12}N_2O_6$.
Nitrogen	15.1		15.2	Molecular weight: 184.15.
Hydrogen		6.6	6.6	Theoretical Oxygen Equivalent: 0.434.
Oxygen		52.4	52.1	Experimental Oxygen Equivalent [1]: 0.434.

[1] From table 5.

Equation 3 is solved for %H, and %O is 100 − (%H + %C + %N).

The determination of the ultimate composition of ammonium tartrate by this method gave the results shown in table 10. Analytical values were taken from table 5. The accuracy in this example is within 1 percent of the true values. The method is capable of good precision and accuracy because it employs true constants, and the assumptions reside in the analyses (completeness of oxidation, accuracy of C and N analyses, correct designation of oxidation end products). This method is usable on most natural samples even though several trace organic elements are disregarded. For example, the difference between the determined (assuming complete oxidation and accurate C and N analyses) and theoretical hydrogen content for protein containing 1 percent sulfur (values from Krogh and Berg, 1931) is only 0.08 percent absolute (i.e., 7.00 percent vs. 7.08 percent H). A nomogram can be prepared from equation 3 to facilitate the calculation of hydrogen, according to the direction in Levens (1948).

CONCLUDING REMARKS

Of the three simple, direct conversions of an Oxygen Consumed datum that have been described, the estimation of organic energy (gcal. = mg. O × 3.4) is probably the most valid and widely applicable. Three transformations employing more than one analytical datum have also been considered. Among these, the determination of proximate composition from the experimental Oxygen Equivalent and organic nitrogen estimate appears to be the most useful. These six derivations fulfill the objectives of this study but do not fully explain the uses of oxidative analysis. The reader is referred to Kuhn and L'Orsa (1931) for a discussion of partial oxidations in the determination of functional groups, and to Williams (1937) for a method of determining the molecular weight of pure organic isolates.

Part VI. LIMNOLOGICAL EXAMPLES OF QUANTATIVE OXIDATION

During the development of the foregoing oxidative techniques, different samples from various sources were investigated to aid in improving the methods as well as to demonstrate their application. Some of these analyses have already been presented, either as specific examples of methods or in support of assumptions. Other analyses have been concerned with a continuing program of regional limnology in the Sierra Nevada area of California.[3] The following three examples represent such pilot studies; their immediate objective is associated with the present research problem but their data are contributory to the continuing limnological program.

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[3] U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, project: California-Nevada Sport Fishery Investigations with headquarters in Reno, Nev.

TOTAL ORGANIC CONTENT OF ALPINE LAKES

Measurement of the standing crop of net plankton is perhaps the most widely used approach in describing the productive status of waters. However, when a vertical plankton tow from a depth of 270 feet yields no detectable catch, the task of making within-lake or between-lakes comparisons becomes rather formidable. This condition of alpine oligotrophy has been observed by Reimers et al. (1955). It is suggestive of the problems encountered in the measurement and interpretation of alpine productivity which originally prompted this study. The analyses of these waters, therefore, afford a prime example of the application of quantitative oxidation in limnological investigation.

Selected descriptive features and analytical data for four lakes in the Convict Creek drainage of the Sierra Nevada are given in table 11. These lakes lie within a circle about 2 miles in diameter and border within 1.5 miles of the principal crestline. Their oligotrophic nature is induced primarily by extrinsic factors related to altitude. Net plankton was not found in two lakes (Dorothy, Cloverleaf) during the initial survey in 1950. More extensive limnological descriptions of these lakes appear in the published survey report (Reimers et al., 1955).

TABLE 11.—*Limnological features and results of oxidative analyses of four lakes in the Sierra Nevada, Calif.*

A. Descriptive features

[Data from Reimers et al. (1955)]

Lake	Elevation (feet)	Area (acres)	Depth (feet)			Milligrams per liter	
			Maximum	Mean	Secchi	Total solids	Hardness
Cloverleaf	10,310	11.6	25	12	bottom	20	9.0
Bighorn	10,675	6.5	35	16	bottom	47	30.0
Dorothy	10,340	152	290	136	67	19	11.0
Edith	10,100	18.0	110	59	46	23	10.0

B. Sampling and analytical data

[Lakes sampled during August 1958]

Lake and location [1] and depth (feet)	Temperature (°F.)	Number of analyses	Milligrams per liter		Gram calories per liter
			Oxygen Consumed	Organic matter	
Cloverleaf:					
NW.–C–3	44	4	1.4	1.22	
NE.–C–3	45	4	1.6	1.40	
SW.–D–3	48	4	1.8	1.58	
SE.–C–3	49	4	2.1	1.84	
Mean				1.5	7.3
Bighorn:					
D–3	58	2	1.2	1.05	
D–15	54	3	1.3	1.14	
D–30	42	2	1.3	1.14	
Mean				1.1	5.4
Dorothy:					
D–15	53	5	2.1	1.84	
D–35	50	5	1.7	1.49	
D–100	39	5	1.9	1.73	
Mean				1.7	8.1
Edith: C/D–3	53	5	1.8	1.58	7.7

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[1] Code letters C and D refer to sampling positions under the center of the lake surface and over the deepest point of the basin, respectively. The four arms of Cloverleaf Lake are listed by compass directions (NW., etc.).

Samples of unfiltered water were analyzed before the presently described procedures were adopted, but the only important departure from the micro method was a shorter reaction time (1.5 to 2 hours). Because of this, the oxidations were less complete and somewhat less precise than those described in part IV. Oxygen Consumed values in table 11 are unadjusted, but the derived values for organic matter and calories have been adjusted for 80-percent complete oxidation. All O.C.'s. are averages of replicate determinations and are significant to within ±8 percent where three or more analyses are combined. Absolute accuracy does not directly concern the comparison here, since all samples were analyzed similarly. Raw water includes both particulate and dissolved substances, but as the data of Birge and Juday (1934) have indicated, the dissolved fraction is strongly predominant.

The average organic matter and caloric values for each lake are not true means (weighted according to the volume at each stratum). These data also give no indication of temporal fluctuations, but the report of Birge and Juday (1927) indicates that such changes are small. The following conclusions should be viewed in the light of the above restrictions.

1. General conclusions. Total organic content of these alpine lakes apparently does not exceed 2 mg. or 10 gcal./L. Even if the assumed correction for incomplete oxidation is in error, it is unlikely that the maximum organic content of any sample is more than 3 mg./L., placing these lakes below the organically poorest ones studied by Birge and Juday (1934).

2. Within-lake comparisons. Cloverleaf Lake is extremely irregular and each of its four arms yields a different analytical quantity which correlates directly with temperature. Vertical differences in organic distribution are implied in Lake Dorothy by an intermediate minimum occurring within the three strata sampled. Significant vertical differences are not apparent in Bighorn Lake, despite a rather strong thermal gradient.

3. Between-lakes comparisons. Depth, as a characteristic of oligotrophy, does not seem to limit organic accumulation (compared with shallowness) for lakes in the same locality, as indicated by the "high" oxidation values of Lake Dorothy. On the other hand, greater depth may induce the accumulation of organic matter in alpine lakes so far as it affects the lake volume relative to rate of change of watermass. Total mineral content, indicated by total dissolved solids, apparently is not related to the organic status of solute-poor lakes. Although Bighorn Lake is highest in total dissolved solids and lowest in organic content, an inverse relationship between these factors should not be assumed. The nature of inorganic ions may be influential inasmuch as this lake has a predominant sulfate anion, while the other lakes are bicarbonate.

QUANTITATIVE PERIPHYTON ANALYSIS

The community of microorganisms which occurs on the surfaces of submerged objects, commonly termed periphyton, has been the subject of various studies (Cooke, 1956). Its accumulation on a given surface area of clean glass slides during the course of a predetermined time interval has been used as an index for comparing the productivity of Sierra Nevada lakes (Nielson, 1953). This quantitative measurement of periphyton growth is accomplished by simple gravimetry. Material removed from dried slides is weighed, ashed, and reweighed to estimate organic matter as ignition loss. One procedural disadvantage is the tedious task of removing periphyton from the slides. A second disadvantage, especially in studies on alpine lakes, is that the amount of matter formed on the slides is frequently too small to be weighed accurately. Oxidative analysis seemed to be a likely way of obviating these disadvantages and was therefore tested by a direct comparison to the gravimetric analysis of periphyton.

Accordingly, one slide (1 × 2 inches) for oxidative analysis was included with each normal gravimetric series of four slides (1 × 3 inches) on sets made in Convict Lake, Calif., in November 1958. Two sets were made: One over the deepest point in the lake consisted of 13 series of slides spaced at 10-foot intervals from 5 feet below the surface to a depth of 125 feet, and a second set of 3 series was placed in shoal water 30 feet deep. The sets were initiated at the onset of fall overturn and were retrieved after 25 days. The four large slides in each series were processed gravimetrically,[4] and the fifth was analyzed oxidatively by the micro method.

The slides were preserved in a dry state and were oxidized by introduction directly into the reaction mixture. It was necessary to reduce the volume of 0.05 *N* dichromate to 2.0 ml. for slides from the deepest water because of their low organic complement. Analytical results were converted to organic weights and to gram calories per square decimeter of slide surface area, assuming the oxidation to be 85 percent complete. Data from both gravimetric and oxidative analyses are plotted in figure 5 with a depth-temperature curve for the lake which was measured during the periphyton growth period. Additional limnological data for Convict Lake are given by Reimers et al. (1955) and the reader is referred to Nielson (1953) for a description of periphyton apparatus and procedure for gravimetric analysis.

A curve has been roughly fitted to the oxidative values in figure 5, and horizontal scales for both organic weight and energy are given. Gravimetric data are shown only for the deep-water set. While oxidative data can be assigned to both scales, energy units are more appropriate. The two methods of estimating periphyton quantity show good general agreement, despite the much smaller surface area (one-sixth) analyzed by oxidation. The values for each depth stratum should not necessarily coincide because one is a direct estimate of mass and the other is more nearly an estimate of energy. The difference between the two estimates in the shallower strata (above 30 feet) may represent a departure from the fixed relation of mass to energy that is assumed here (1 mg. = 5 gcal.).

The time required for the oxidative analyses was probably less than half that needed for the gravimetric analyses. Since values obtained by the latter method are not necessarily more accurate estimates of organic mass, it is concluded that the oxidative measurement of periphyton is equal, if not superior to gravimetry. It has the added advantages of sensitivity and ease of analysis, and the potential benefits of elemental (C and N) estimates.

Interpretation of the periphyton data in figure 5, apart from the methodological comparison, suggests some interesting limnological relationships. This study does not attempt to relate periphyton production to the entire lake biota, or to define rigidly its trophic status. It is assumed here that the periphyton of Convict Lake is primarily an autotrophic population (see footnote 4) quite similar in structure to nannoplankton. This is the basis for the following observations:

1. The deep-water curve indicates a logarithmic decrease in periphyton abundance with depth, analogous to a

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[4] These determinations were made by H. D. Kennedy, Convict Creek Experiment Station, Bishop, Calif. Mr. Kennedy also tentatively identified most of the periphyton organisms, which are almost exclusively algal (54 genera of algae observed, 30 of them diatoms).

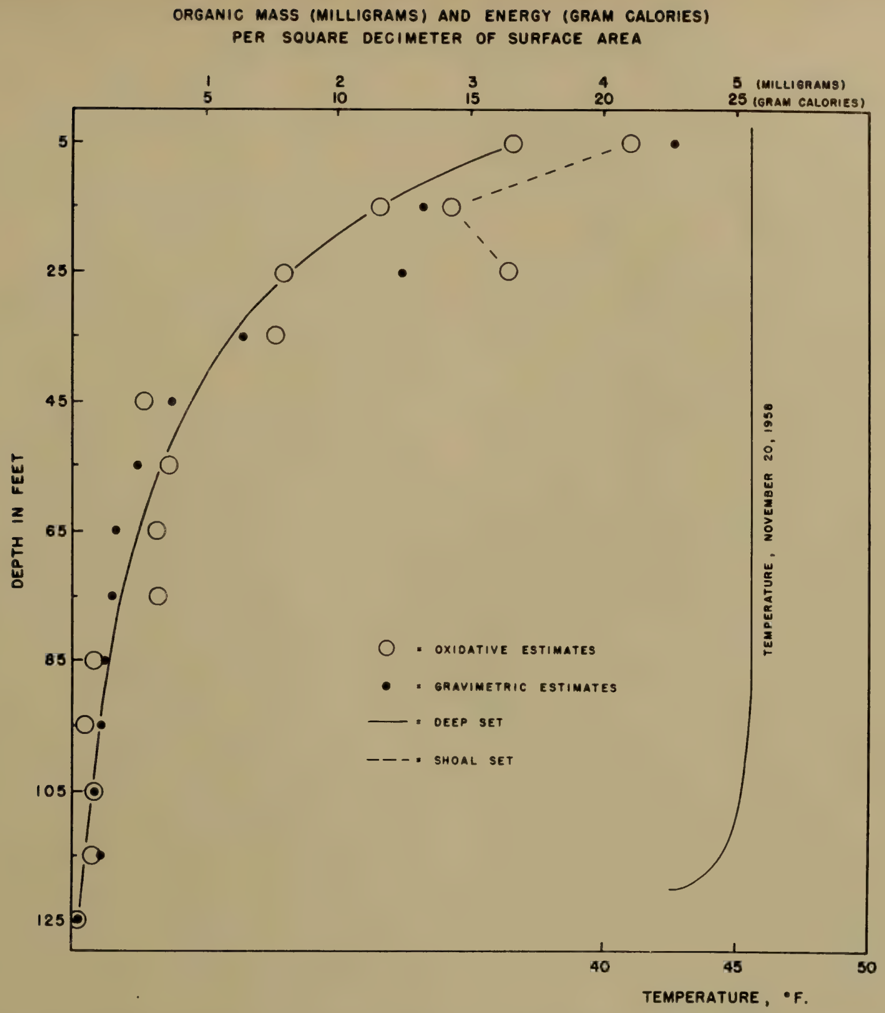

FIGURE 5.—Periphyton growth in Convict Lake, Calif., during a 25-day period (November 4–29, 1958) as determined by oxidative and gravimetric methods.

light penetration curve. Since the sampling is coincident with fall overturn, the effects of temperature and nutrient distribution are minimized. Therefore, the growth of periphyton at all depths seems dependent upon radiation intensity, and independent of vertical changes in population composition.

2. An increase in periphyton quantity is noted in the lowermost series of the shoal set. That this phenomenon is real is supported by other similar observations. This "bottom reinforcement" is probably not caused by nutrient diffusion from the sediments, but since light is evidently limiting, it is more likely attributable to a reflective increase in radiation intensity. A complex pattern of autotrophic production in the vertical dimension is suggested for the shallower waters of deep, clear lakes (or throughout shallow lakes) under conditions of light dependency, especially where bottom materials are reflective. The primary production of such a lake, induced from one or a few static vertical series of measurements, may carry a significant assumptive error.

3. A consideration of the two types of curves is indicative of the difficulty to

be expected in comparing periphyton production (rates) of lakes differing in depth and area. On the other hand, quantitative periphyton study can be a means of investigating the influence of spatial distribution and lake morphometry on a producing population.

PLANKTON AND NEUSTON COMPOSITION

Two samples of plankton were taken from Crowley Lake, California for the purpose of determining their nutrient value. One of them is a relatively pure sample of the blue-green alga *Aphanizomenon* and the other is a similar sample of the cladoceran *Daphnia.* A third sample, representing a mixed population of microorganisms, is neuston material from a pond surface near Cortland, N. Y. All of these substrates are of limnological importance insofar as they contribute to autochthonous abioseston and dissolved organic matter upon disintegration or decomposition. The neuston material is probably of little significance in lakes but its composition apparently has not been investigated. These samples have aided in the development of the methods previously described and their Oxygen Equivalent values were shown in tables 3 and 6. The following determinations are more complete and independent of those given earlier. They are presented as a summarial demonstration of the nature and amount of information obtainable from the "complete" dichromate oxidation technique.

Analyses follow the procedures in part IV (semimicro method of oxidation with carbon and nitrogen determinations), and the interpretations of part V. The results are given in table 12. Oxygen Consumed values are lower than those previously noted because the samples had gained a few percent more moisture during storage. Oxygen Equivalents are adjusted for 5-percent incomplete oxidation, and carbon data for 3-percent incomplete recovery. The proximate composition of *Daphnia* has been calculated with and without an allowance for the distribution of nitrogen between chitin and proteinaceous materials. Observations on the derived data are summarized below.

1. The three substrates are similar in general organic respects; their O.E.'s. are close to the "average" for limnological samples.

2. The elemental percentages occur within the general range given for plankton and seston by Birge and Juday (1922), Brandt and Raben (1920), and Krogh and Berg (1931). Carbon contents are suspected to be slightly lower than their true values, perhaps due to incomplete recovery apart from incomplete oxidation.[5] Neuston nitrogen is definitely a low percentage for biological materials and may reflect a composition difference for organisms existing at the water surface ecotone.

3. Proximate composition varies more among the substrates than ultimate composition, and is therefore more distinguishing. Neuston is low in crude protein and high in crude carbohydrate and lipid, which is related to the observation above. *Daphnia* is highest in crude protein and lowest in crude carbohydrate, as expected, but is lower in lipid than other reported zooplankton analyses (see table 8).

The actual application of data such as these will depend on the nature of the study undertaken, but they are expected to be of value throughout the limnological field. The salient and concluding issue here is that a great deal of information is available from a few relatively simple analyses based on quantitative oxidation. All of the fore-

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[5] A semi-independent comparison can be made by summing the assumed carbon contents of the proximate groups. Carbon percentages calculated in this manner are *Aphanizomenon* 49 percent, *Daphnia* 47 percent, and Neuston 51 percent.

TABLE 12.—*A chemical characterization of three samples of aquatic organisms by oxidative analysis*

Substrate	Analytical data (air-dry weight basis) [1]							Derived data (moisture-and ash-free basis) [2]								
	Sample data		Oxygen Consumed (mg. per mg. sample)	Percentage found			Oxygen Equivalent	Ultimate and proximate percentages							Energy, kilocalories per gram	
	Number analyzed	Weight range (mg.)		Carbon	Nitrogen	Ash-free dry weight		Carbon	Nitrogen	Hydrogen	Oxygen	Crude protein	Lipid	Crude carbohydrate	Sample	Carbon
Aphanizomenon	4	22.5–26.9	1.191	40.4	6.83	90	1.41	46.6	7.6	8.1	37.6	47.6	4.6	47.8	4.80	10.3
Daphnia	5	19.0–31.2	0.988	32.0	7.13	71	1.46	46.8	10.1	9.0	34.1	63.0	4.6	32.4	4.97	10.6
												[3] 54.1	[3] 6.8	[3] 39.1		
Neuston	4	20.5–21.9	1.094	39.1	4.34	80	1.43	50.3	5.4	6.7	38.6	33.6	8.7	57.7	4.86	9.7

[1] Analytical results are unadjusted means.

[2] Oxygen Equivalents adjusted for 5-percent incomplete oxidation, percentage carbon for 3-percent incomplete carbon recovery. Caloric values do not include energy of nitrogen oxidation, which can be calculated (see part V).

[3] Adjusted for 20 percent chitin, a value assumed from Geng (1925).

going data for any sample may be determined from a single "complete" oxidation, assuming the organic content of the sample is known. The entire analytical procedure requires one weighing, one volumetric dilution, 3 accurate pipettings, and 3 titrations for which only 2 standardized reagents are required.

Part VII. SUMMARY

A scheme is presented for the characterization of natural organic matter which is based on its reaction with acid dichromate. Conditions of strong acidity and prolonged heating enable dichromate to oxidize nearly all organic compounds effectively, returning substituent elements to normal inorganic states. This reaction provides a basis for three analyses. The principal analysis is a measurement of the amount of reacting oxygen (quantitative oxidation) and is described in two related procedures: the semimicro method, applicable mainly to weighable particulate samples; and the micro method, designed especially for minute amounts of organic matter in deciliter volumes of water. The other two analyses concern the diffusion separation and estimation of organic carbon and nitrogen as reaction end products (CO_2 and NH_3). All analyses are accomplished titrimetrically, and emphasis is placed on procedural simplicity.

These analyses apply broadly to limnological samples, based on an evaluation of organic oxidation states. Natural organic matter is considered a mixture of compounds in which carbon is reduced from the dioxide by bonding which excludes some oxygen from the molecules. The extent of this reduction is distinctive for different compounds and chemically similar classes. The amount of oxygen required for reoxidation (Oxygen Consumed) is related to the energy, mass and composition of sample organic matter. These relationships are summarized in the following diagram (fig. 6). The limnological application of this analytical scheme is demonstrated with samples of plankton, periphyton and alpine lake water.

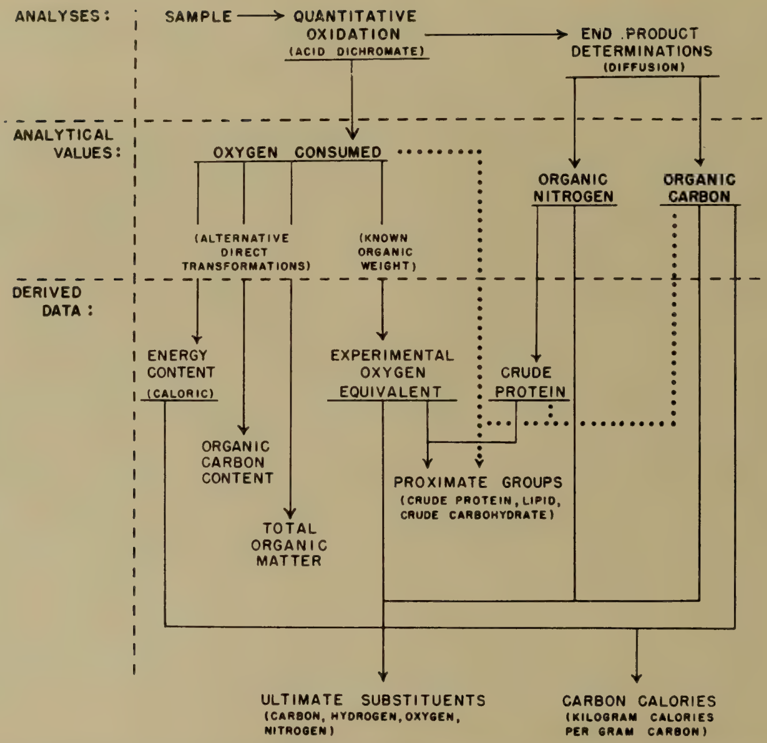

FIGURE 6.—Flow diagram of the salient features of organic analysis by quantitative oxidation.

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APPENDIX—PROXIMATE COMPONENTS IN LIMNOLOGICAL SAMPLES

The proximate components (carbohydrates, fats, and proteins) can be considered as groups of chemically related compounds. These component groups have been used in characterizing limnological organic matter by Birge and Juday, Krogh and coworkers, and many others. Carbohydrates, fats, and proteins, as described and discussed in general biochemical texts, are quite distinct entities, but analytical partitioning of total sample organic matter into three proximate groups necessarily places some less closely related compounds together. It is improper, then, to consider carbohydrates only as hexoses or hexose polymers, fats only as fatty acids or triglycerides, and proteins only as amino acids or their condensates. To emphasize this difference, the analytical proximate groups are referred to here as crude protein, lipid, and crude carbohydrate.

The most common proximate analysis determines lipid by organic solvent extraction, crude protein by conversion of the measured organic nitrogen, and crude carbohydrate as the amount of residual matter. Obviously, organic nitrogen is present in compounds other than proteins or their constituent molecules, and solvent extraction removes some nonfatty materials. Residual "carbohydrate" then contains all compounds not otherwise accounted for, as well as the analytical errors of the other two determinations. It is felt that these proximate groups would have greater significance and utility in limnological studies if they were more closely defined in terms of the most important compounds actually present. Furthermore, it is necessary for the analytical scheme presented here to evaluate the more important oxidative characteristics of the proximate groups and interpret them as representative values. In doing this, it is desirable to view the proximate groups as they are determined by the more common analytical techniques so that the results of oxidative analysis will be compatible with data of other research.

The characterization of proximate components has been accomplished by arranging the most abundant limnological compounds (or representatives) and their characterizing data into groups and subgroups (appendix tables A–1 through A–5). Empirical formulas and energy contents (kcal./g. substrate) were taken mainly from Hodgman (1959) and Washburn (1929). From these data, the percentages of carbon and nitrogen, the Oxygen Equivalents (text, p. 7), and the other energy relations were calculated. The most useful energy value in oxidative analysis is kilocalories per gram of oxygen consumed (or gcal./mg. O.C.), which Vinberg et al. (1934) have referred to as the "oxycalorie coefficient." Calorie values for nitrogenous compounds do not include energy of nitrogen oxidation (see text, p. 39). This compilation and its interpretation are based on the works of Fogg (1953), Haurowitz (1955), Vallentyne (1961), and especially Vallentyne (1957), with lesser references noted in context. A great many compounds which would be included in this classification chemically, have not been demonstrated as quantitatively important in limnological material. On the other hand there are doubtless several prominent natural compounds which deserve inclusion but remain to be described chemically. The listed compounds are assumed to be sufficient in number and type to permit a satisfactory introductory characterization of proximate groups. This characterization is a tentative personal interpretation of existing information and may be modified by better quantitative molecular information as it becomes available.

Crude carbohydrate

A classification of carbohydrate materials is given in appendix table A–1. All of these compounds are susceptible to rapid oxidation by dichromate and, lignoids excepted, are characterized by low Oxygen Equivalents, carbon contents, and calorific values per unit compound weight, and by high oxycalorie coefficients. "True" carbohydrates appear in the upper half of the table and are considered the most abundant carbohydrate forms in cellular material such as net plankton, macrophytes, nannoplankton and periphyton. Sample material of this type is assumed to contain crude carbohydrate represented by an O.E. of 1.2, an oxycalorie coefficient of 3.5, and a carbon content of 45 percent.

Lignoids and some of the low molecular weight, high oxygen content compounds (especially acids) are expected to occur analytically as carbohydrates because of their insolubilities in organic solvents. Lignin and humic-type acids are classed together as lignoids because of similar properties (cyclic structure, methoxyl groups, high O.E. and carbon content). Abioseston and sediments may contain a large amount of lignoids (Kleerekoper, 1953). Kleerekoper and Grenier (1952) estimated that up to one-half of the organic material at the surface of bottom sediments is lignin. Lignoids are also prominent components of dissolved organic matter especially in humic waters. Although Shapiro (1957) has observed organic solvent solubility for humolimnic isolates, the data of Birge and Juday (1927, 1934) indicate ether insolubility for dissolved humic materials. This difference may be due to different sample pretreatment. It is concluded here that the crude carbohydrate of sedimented and dissolved humic matter differs from that of cellular substance and has the representative values: carbon, 50 percent; O.E., 1.4.

Appendix Table A–1.—*Crude carbohydrates: representative compounds and some of their characteristics important in oxidative analysis*

Classification	Molecular formula	Percentage carbon	Oxygen Equivalent	Kilocalories per gram		
				Substrate	Substrate carbon	Oxygen Consumed
Hexoses and hexosans:						
Glucose	$C_6H_{12}O_6$	40.0	1.06	3.74	9.4	3.51
Starch, cellulose	$(C_6H_{10}O_5)n$	44.4	1.18	4.18	9.4	3.53
Hexosamines:						
Chitin [1]	$C_{30}H_{50}O_{19}N_4$	46.8	1.25	----------	----------	----------
Pentosans:						
Xylan	$(C_5H_8O_4)n$	45.4	1.21	4.26	9.4	3.51
Polyuronides and pectins:						
Pectic acid	$(C_6H_8O_6)n$	40.9	0.91	----------	----------	----------
Related compounds [2]:						
Lignin [3]	$C_{9.9}H_{11.4}O_{3.5}$	63.6	1.89	6.28	9.9	3.32
Humic and crenic acids	(see table 7)	49–55	1.48–1.55	----------	----------	----------
Pyruvic acid	$C_3H_4O_3$	40.9	0.91	3.16	7.7	3.49
Malic acid	$C_4H_6O_5$	35.8	0.72	2.39	6.7	3.34

[1] Composition from Strecher (1960).

[2] Examples of various compounds which may occur in this group.

[3] Data from Brauns (1952).

Lipid

Various strongly reduced, high-carbon compounds with high Oxygen Equivalents are grouped in appendix table A–2 as lipids. They are generally soluble in organic solvents, and slowly oxidized because of their immiscibility with water. Fats and fatty acids predominate in cellular material. The hypothetical triglyceride shown is based on the most abundant algal fatty acids listed by Fogg (1953). In addition to fats, extracted algal lipid may contain up to 40 percent pigments (Burlew, 1953); of these, chlorophylls are present in largest amounts (Hill and Whittingham, 1958). Free fatty acids observed in lake water (Goryunova, 1952, 1954a) were attributed to secretions from diatoms and other algae (Goryunova, 1954b). Fatty acids, pigments and their degradation products, and waxes are known from seston and sediments (Vallentyne, 1957).

The four subgroups in the upper portion of appendix table A–2 represent the most reduced states of these compounds. As decomposition or degradation proceeds, especially with fatty acids and porphyrins (pyrrole compounds), carbon contents and O.E. values decrease. Organic solvent extraction can be expected to remove some nonlipid material from a sample, and this occurrence is noted as the final group in the table. In contrast to the lipids, these compounds are of low carbon content and O.E., and more properly belong to the crude carbohydrate group.

Except in some living cells (such as diatoms or zooplankters) the total sample lipid does not exceed about 10 percent. This fact makes lipid the least abundant of the proximate groups. Nevertheless, it has a strong influence on the sample O.E. and carbon content by virtue of its highly reduced state. The characteristic values assumed here for limnological lipid are carbon 70 percent, O.E. 2.7, oxycalorie coefficient 3.3.

Crude protein

The nitrogen-containing materials include the most important biological compounds and frequently comprise the largest fraction of sample organic matter. However, the group appears to be poorly understood in proximate analysis of limnological material and is therefore given special consideration.

Amino acids and the larger molecules into which they condense are considered "true" proteins. Elemental analyses of purified proteins indicates that they are very nearly 16 percent nitrogen, and it is from such "text" values that the conversion factor for estimating crude protein is obtained. Individual amino

APPENDIX TABLE A–2.—*Lipids: representative compounds and some of their characteristics important in oxidative analysis*

Classification	Molecular formula	Percentage carbon	Oxygen Equivalent	Kilocalories per gram		
				Substrate	Substrate carbon	Oxygen Consumed
Fats, oils and fatty acids [1]:						
Palmitic acid	$C_{16}H_{32}O_2$	75.0	2.86	9.36	12.5	3.26
Palmitolic acid	$C_{16}H_{28}O_2$	76.2	2.79	---	---	---
Stearolic acid	$C_{18}H_{32}O_2$	77.1	2.85	9.38	12.1	3.29
Triglyceride (of the above)	$C_{53}H_{94}O_6$	76.9	2.85	---	---	---
Pyrrole compounds:						
Chlorophyll (pheophytin)	$C_{55}H_{74}O_5N_4$	75.9	2.50	---	---	---
Carotenoids, isoprene derivatives:						
Beta-carotene	$C_{40}H_{56}$	89.9	3.23	---	---	---
Sterols and steroids:						
Fucosterol	$C_{29}H_{48}O$	84.3	3.13	---	---	---
Related compounds [2]	Various	50	1	5	10	3.3

[1] Molecules assumed to be representative from the data given by Fogg (1953).

[2] Generalized values for organic solvent soluble, oxy- and hydroxy-compounds which may occur in this group.

APPENDIX TABLE A–3.—*Characteristics of some free amino acids from aquatic plant and animal protein hydrolyzates*

Amino acid	Molecular formula	Percentages		Oxygen equivalent	Kilocalories per gram		Order of abundance [1]		
		Carbon	Nitrogen		Amino acid	Oxygen consumed	Algae	Invertebrates	Brook trout
Leucine and isoleucine	$C_6H_{13}O_2N$	55.0	10.7	1.829	5.92	3.24	1	1	1
Glutamic acid	$C_5H_9O_4N$	40.8	9.5	0.979	3.15	3.22	3	2	2
Aspartic acid	$C_4H_7O_4N$	36.1	10.5	0.721	2.31	3.20	5	3	5
Lysine	$C_6H_{14}O_2N_2$	49.2	19.1	1.530	5.09	3.32	6	5	6
Alanine	$C_3H_7O_2N$	40.4	15.7	1.077	3.48	3.23	8	7	4
Valine	$C_5H_{11}O_2N$	51.2	12.0	1.640	5.29	3.23	2	9	8
Arginine	$C_6H_{14}O_2N_4$	41.4	32.1	0.989	3.49	3.45	7	4	9
Proline	$C_5H_9O_2N$	52.1	12.2	1.529	5.09	3.33	4	8	10
Glycine	$C_2H_5O_2N$	32.0	18.7	0.639	2.08	3.25	11	14	3

[1] Based on data given by Fogg (1953) and Block (1959).

acids, as shown in appendix table A–3, actually contain quite dissimilar nitrogen percentages. However, the average contents of nitrogen for aggregates of free amino acids or for proteins are much less variable. Other characterizing values also can be represented by averages. Thus, amino acid carbon content is about 50 percent, Oxygen Equivalent is about 1.5, and energy content is about 5 kilocalories per gram. Oxcalorie coefficients, averaging nearly 3.3, vary the least. The above values increase slightly when calculated for combined acids because one water molecule is lost for each peptide bond formed. The calculated O.E.'s and energy contents of several proteins are given in appendix table A–4. On the whole, they are quite similar (O.E. 1.49 to 1.60; kcal./g. 4.92 to 5.29) but a distinct difference is evident between the algal and animal proteins, the former having a higher average O.E. and caloric content.

APPENDIX TABLE A–4.—*Oxygen Equivalents and caloric values of some aquatic plant and animal proteins*

[Based on compositions given by Block (1959) and Fogg (1953)]

Protein source	Oxygen equivalent [1]	Kilocalories per gram of protein [2]
Algae:		
Anabaena (Cyanophyta)	1.58	5.21
Chlorella (Chlorophyta)	1.56	5.15
Navicula (Bacillariophyta)	1.59	5.26
Tribonema (Chrysophyta)	1.60	5.29
Aquatic invertebrates:		
Gammarus (Crustacea)	1.49	4.92
Ephemeroptera (Insecta)	1.53	5.06
"Mixed insects" [3]	1.52	5.02
Trout (*Salvelinus*):		
Wild	1.49	4.92
Hatchery	1.49	4.92

[1] Calculated by adjusting all amino acid percentages reported for each source to 100 and summing their individual weighted Oxygen Equivalents (combined acid O.E. = free acid O.E. × mol. wt./mol. wt. — 18).

[2] Calculated from Oxygen Equivalents and oxycalorie coefficients (kcal./g. = O.E. × 3.3). Corresponding calorimeter values would be about 0.88 kilocalories per gram higher.

[3] Chiefly Plecoptera.

In some biological materials, the assumption that total organic N is reckonable as protein may be very nearly correct (Haurowitz, 1955, noted that less than 2 percent of human blood nitrogen is nonprotein). Limnological samples may be strikingly different, and this assumption can cause a rather misleading conception of crude protein. Nitrogenous compounds have been listed in the two previous groups (carbohydrate chitin and lipid pyrroles). Others are given in the lower part of appendix table A–5. These nonprotein nitrogenous compounds have different characteristic values (especially percentage N and O.E.) and, if present in a sample in substantial amounts, may cause a significant error in the crude carbohydrate estimate. The calculation of crude protein (organic N × 6.25) mathematically assigns at least some of any nitrogen-containing material to this group. Geng (1925) has shown that invertebrates may contain up to 20 percent chitin (properly, a carbohydrate). The

APPENDIX TABLE A–5.—*Crude proteins: representative compounds and some of their characteristics important in oxidative analysis*

Classification	Molecular formula	Percentages		Oxygen equivalent	Kilocalories per gram		
		Carbon	Nitrogen		Substrate	Substrate carbon	Oxygen consumed
Proteins [1]:							
1	$C_{4.32}H_{7.00}O_{1.50}N_{1.14}$	52	16	1.44	4.8	9.2	3.3
2	$C_{4.45}H_{7.00}O_{1.49}N_{1.11}$	53.5	15.6	1.48	---	---	---
Amino acids	(see table A-3)	32–55	10–32	0.6–1.8	2–8	---	3.3
Related compounds [2]:							
Hexosamines:							
Chitin	$C_{30}H_{50}O_{19}N_4$	46.8	7.4	1.24	4.3	9.3	3.5
Pyrrole compounds:							
Pheophytin	$C_{55}H_{74}O_5N_4$	75.9	6.4	2.50	---	---	---
Nucleic acid derivatives:							
Adenine	$C_5H_5N_5$	44.5	51.9	.59	---	---	---
Uracil	$C_4H_4O_2N_2$	42.9	25.0	.71	---	---	---
Excretory products:							
Uric acid	$C_5H_4O_3N_4$	35.7	33.3	.29	.91	2.5	3.2

[1] Data for protein No. 1 from Krogh and Berg (1931) and for protein No. 2 from Brandt and Raben (1920). See appendix table A–4 for other values.

[2] Examples of nitrogen-containing compounds placed in this group by analytical definition.

aforementioned calculation partitions chitin between the two proximate groups; an example of this error is demonstrated in text table 12. On the other hand, if strongly nitrogenous excretory products are present (such as urea or uric acid) the conversion error places an excess quantity of matter into the crude protein group and diminishes the crude carbohydrate proportion which is calculated by difference. The latter effect cannot be demonstrated by existing data, but must surely occur to some extent on occasion. Because crude protein by analytical definition is organic N × 6.25, these errors cannot be resolved easily until more is known about the molecular nature of limnological organic-nitrogen compounds.

Cellular material can be expected to contain a predominance of protein nitrogen. The studies of Domogalla et al. (1925) and Peterson et al. (1925) have indicated that most dissolved organic N is proteinaceous (amino and peptide). More recently, Vallentyne and others (see Vallentyne, 1957) have identified several amino acids from hydrolyzates of dissolved and sedimented organic materials. Other important nitrogenous compounds are poorly characterized.

The data given for proteins, amino acids, and nonprotein nitrogenous compounds in appendix table A–5 suggest the following representative values for crude protein: Carbon, 52 percent; O.E., 1.5; oxycalorie coefficient, 3.3. This O.E. is possibly too high for dissolved matter, and for particulate matter where living plant cells are not abundant. A crude protein O.E. of 1.45 or 1.4 may prove to be more appropriate in such instances.

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BIOASSAY AND USE OF PITUITARY MATERIALS TO SPAWN WARM-WATER FISHES

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 61

RESEARCH REPORTS

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BIOASSAY AND USE OF PITUITARY MATERIALS TO SPAWN WARM-WATER FISHES

Howard P. Clemens and
Kermit E. Sneed

RESEARCH REPORT 61

UNITED STATES DEPARTMENT OF THE INTERIOR
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FISH AND WILDLIFE SERVICE
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Daniel H. Janzen, *Director*

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ABSTRACT

By bioassay, evaluation was made of the activity and relative potency of pituitaries collected throughout the year from various species of fish. Goldfish, zebra fish, and channel catfish were the chief test species; carp, green sunfish, largemouth bass, white crappie, and flathead catfish were used occasionally. Positive response was indicated by ovulation of eggs or by increase in seminal plasma.

In experiments to determine the species from which pituitaries can be used to spawn various species (phylogenetic specificity), 29 interspecific and intergeneric injections and 25 interfamilial injections were successful; only 7 heteroplastic injections failed to induce ovulation or exhibited low activity. Of the 7 failures, 4 involved river carpsucker and fresh-water drum pituitaries at regular dosage levels; and the other 3 were unsuccessful attempts to spawn carp with other than homoplastic pituitaries. Specificity appears so slight that it has no practical importance in fish-cultural techniques.

Activity of pituitaries collected in various months did not vary as much as the individual variations in physical condition of the test fish. The data indicate that all the pituitaries are active in the months in which collections were made. Activity in relation to size, sex, and degree of gonadal development of the donor fish does not appear as important as implied by some previous reports in the literature.

No detrimental effects of pituitary injections have been detected in incubating and hatching the eggs, or in rearing the fingerlings, from over 200 channel catfish spawns and thousands of goldfish spawns.

BIOASSAY AND USE OF PITUITARY MATERIALS TO SPAWN WARM-WATER FISHES

By **Howard P. Clemens**
University of Oklahoma
Norman, Oklahoma

and **Kermit E. Sneed**
Southeastern Fish Cultural Laboratory
Marion, Alabama

Use of pituitary materials to induce fish to spawn has many advantages in the culture of sport, food, bait, and tropical fishes. Since fully ripe females of most species respond to pituitary injections by spawning within 12 to 20 hours, it is possible to predict rather accurately when a spawn will occur. This predictability allows the fish-culturist to plan his work according to a rigid schedule, since the injections, to some degree, bypass the variables in the environment, such as temperature, light, and rain. Since both males and females respond positively to pituitary injections, most species can be hand-stripped, a practice which offers additional advantages. Culture ponds can be stocked with eggs and fry of uniform age and size. Since the brood stock are not in the pond with the offspring, the possibility of transmission of disease from brood stock to offspring is reduced, and predation by the parent is eliminated. Hybrids can also be produced by hand-stripping whenever hybridization is desirable and feasible. Wild fish that have not yet been domesticated may be spawned more easily by the pituitary method. Frequently more uniform spawning has been achieved by the injection of pituitary materials into ripe fish which, because of some environmental or physiological condition, would fail to spawn without such treatment.

South American and Russian fish-culturists have applied the pituitary method to the large-scale spawning of fish of economic importance. In the United States such work has been limited for the most part to small experimental lots of fish. This is pointed out by Pickford and Atz (1957), who reviewed and discussed all the available literature on the use of pituitaries. A study of their book, indispensable to anyone interested in fish gonadotropins and their use in fish culture, reveals: (1) Fish pituitaries contain the hormones to precipitate spawning in ripe fish. (2) There is little if any specificity in the hormones of fishes; that is, the pituitaries of one family or species are usually active in unrelated species. (3) There are a number of examples in which gonadotropic materials from other vertebrate classes were found to be active in fish. (4) Out-of-season spawning has been induced by the administration of gonadotropins. (5) Fresh, acetone-dried, or frozen pituitaries, as well as glycerine extracts, saline suspensions, and water extracts of pituitaries have similar activity. (6) Under most experimental conditions the injection of pituitaries does not decrease fertility. (7) Increased or de-

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creased exposure (depending on species of fish involved) to light may have the same effect on sexual development and spawning as pituitary injections. (8) The lack of response in some experiments with mammalian gonadotropins may be due to the fact that the dosage was too low (Kawamura and Otsuka, 1950, used quite high levels to achieve positive results). Also, fish may require a ratio of components different from that contained in some mammalian materials; and there may be a quantitative difference in the ratio of hormones rather than a qualitative difference in the hormones themselves. (9) There is little or no qualitative difference in the pituitaries of the male and female donor fish, at least from a practical viewpoint. (10) There may be a "seasonal" difference in the biological effect produced by pituitaries taken from fish prior to spawning and those taken immediately after spawning.

Much more must be learned before the qualitative and quantitative differences in fish pituitaries at various times in the year are known. The responses to these pituitaries of fish of different physiological condition, age, and development must also be determined. At present, good bioassay methods for fish gonadotropins are unknown. Witschi (1940) demonstrated that the LH component of vertebrate pituitaries could be assayed relatively by injecting the material into weaver finches. The method for the bioassay of FSH in fish pituitary materials involves the cornification of the mouse vagina, but these methods have been questioned by some workers. Until good methods are devised, rather crude bioassay methods of whole pituitaries and unpurified extracts must be used.

BIOASSAY OF PITUITARY MATERIALS

Collection and preparation of pituitaries

There are many potential sources of fish pituitaries. When only a few pituitaries are needed it is usually practical to sacrifice the culls from the brood stock which is to be spawned. Our experience has indicated that these culls have active pituitaries. Also, they are usually similar in size to fish to be spawned and their pituitaries can be injected without weighing or diluting. At some fish hatcheries it is advantageous to have a stock of carp or goldfish to provide a few pituitaries when needed. Often wholesale fish markets may handle fish from which pituitaries can be obtained, provided that the fish are strictly fresh or were frozen when fresh. Pituitaries (mostly from buffalo, *Ictiobus*) that were frozen in the head of fish for a few days up to at least 1 year were effective in spawning goldfish, but these were not as uniform or as potent in action as fresh material. An excellent source of a large number of pituitaries is from the rough fish caught by commercial fishermen or State fish and game department employees when they use traps, nets, or rotenone. Pituitaries which are allowed to remain at high temperatures after the death of the fish probably lose their gonadotropic activity because of enzymatic action.

Our method of collecting the pituitaries was to cut off the heads of the fish with a cleaver, remove the top of the cranium of each immediately above the eyes, and lift out the brain with forceps. In some species the pituitary remained attached to the ventral part of the brain; in other species it was left in the myodom in the floor of the brain case. The pituitary was removed with the aid of forceps. In large-scale collections, 9 men could collect about 1,000 pituitaries per hour. There are some species of fish, such as gar (*Lepisosteus*) and gizzard shad (*Dorosoma*), whose pituitaries are so difficult to remove that collection is hardly worthwhile. The pituitaries of the gar had to be exposed by use of a bandsaw because of the ganoid scales, and the pituitaries of the shad are extremely delicate.

The donor fish (those from which the pituitaries were removed) were always sorted according to species. Unless they were to be used for special experiments, no attention was given to sex, maturity, or size, although fish of sufficient size were chosen to justify the effort of collecting the pituitaries. In most cases, we assumed that the smaller fish in our collection would probably reproduce during the next spawning season.

Since the pituitary gonadotropins are proteins subject to enzymatic deterioration, it is logical to assume that pituitaries frozen or placed in acetone promptly after removal will produce better gonadotropic activity. Some pituitaries were placed in vials in the field and immediately frozen in insulated cans of dry ice. These were later freeze-dried in the laboratory. If acetone was used, the pituitaries were dehydrated in four changes of cold acetone 12 hours apart. Drying-vials should be kept full of acetone so that moisture from the air will not condense on the inside walls of the vials and dilute the acetone. For the same reason, the acetone of the last change should be allowed to warm to room temperature before it is removed and the pituitaries allowed to air-dry.

Pituitaries preserved by either method should be stored in a dry atmosphere, preferably in a dessicator charged with calcium chloride or other drying agent. If the pituitaries are to be used promptly or if the humidity is low, tightly stoppered vials can serve as temporary containers. We have found that pituitaries treated in this manner are potent after 2 years (our longest data period), but Hasler, Meyer, and Wisby (1950) reported that acetone-dried carp pituitaries stored and preserved in this way were active after 10 years.

Dry pituitaries were finely ground and injected into the body cavity as a suspension in distilled water (other diluents, such as physiological saline or isotonic salt solution, theoretically should be less harmful to the tissues, but distilled water combined with dried pituitaries gave acceptable results and appeared to be relatively harmless to the fish). Fresh pituitaries were injected by placing the gland in the socket of the needle and forcing it through the needle stem with distilled water. The site of injection was usually at the axil of the pelvic fin. Intramuscular injections were also given, but the body-cavity injection was more satisfactory. Intramuscular injections are more convenient when the fish are so large that it is cumbersome to remove them from the tank, but such injections are more likely to produce lesions that damage the fish. When injected intraperitoneally the diluent volume can be larger and less critical, and injections can be more easily and quickly administered.

Other carriers as well as distilled water were tested. They were mixed with whole, ground, acetone-dried pituitaries and injected at "full-dose" (0.7 milligram) and "half-dose" (0.4 milligram) levels. One of these, cholesterol, was mixed with ground pituitaries to give a ratio of 300 : 7 at the "full-dose" level and 300 : 4 at the "half-dose" level. This mixture was compacted into cylindrical pellets which were injected with a caponizing needle into the body cavity of the fish. Similar dosages of pituitary materials containing 1 milliliter of distilled water or of sesame oil were injected into the body cavities of other fish. In this experiment female goldfish were divided into groups of four and injected as listed in table 1.

The cholesterol and sesame oil were used to reduce the rate of absorption and to make absorption more uniform over a longer period of time. It appeared that the absorption rates were changed, but the change was not advantageous to our present problem of spawning ripe fish.

Pickford and Atz (1957) reviewed the use of glycerine extracts of pituitaries and pointed out the reported possible advantages of greater gonadotropin recovery, slower absorption, and ease of storage in sealed vials at room temperature. We wish to add that injections of glycerine extracts are easier to administer than aqueous suspensions of pituitaries, but the preparation of these extracts is tedious. De Menezes, Fontenele, and Comacho (1945) injected small volumes (0.5) milliliter) of glycerine extract which contained as high as 66 percent glycerine. The use of this high percentage of glycerine theoretically is biologically inconsistent with the osmotic properties of the body cavity. Since the volume of aqueous suspensions most often used in our experiments was about 1 milliliter, we injected goldfish (*Carassius auratus*) with this volume of water with the following percentages of glycerine: 1, 2.5, 5, 10, 20, 30, 40, 50, 60, and 70. Each injection contained 10,000 units of penicillin, but contained no gonadotropin. All fish that received injections of 10 percent or less glycerine suffered no harmful effects after 3 days at 60° F., while fish that received injections containing more than 10 percent glycerine showed excessive fluid in the body cavity, peritoneal inflammation, and enlarged hemorrhagic areas at the point of injection. On this basis, we decided to keep the glycerine content below 10 percent whenever possible, even though another experiment (table 2) indicated that small volumes of higher percentages of glycerine were suitable.

In a limited experiment with goldfish, injections of equivalent amounts of acetone-dried carp pituitary as aqueous suspensions and as glycerine extract (table 2) showed little difference in effectiveness.

Table 1.—*Female goldfish* (Carassius auratus) *ovulated with dried pituitary in various carriers*

Carrier	Dose	Number injected	Number ovulated
Distilled water	Full	4	4
Do	Half	4	4
Cholesterol	Full	4	4
Do	Half	4	4
Sesame oil	Full	4	1
Do	Half	4	1

Table 2.—*Comparison of effect of injections of aqueous suspensions of pituitaries and glycerine extract in female goldfish* (Carassius auratus) *at water temperature of 60° F.*

Dosage level (equivalent acetone-dried carp pituitaries)	Volume of injections (milliliters)	Ratio of ovulating females to number injected		
		Aqueous suspension	75-percent glycerine extract	84-percent glycerine extract
0.0 milligrams [1]	1. 0	0:3	0:3	0:3
0.2 milligrams	0. 1	1:3	3:3	1:3
0.5 milligrams	0. 25	3:3	3:3	2:3
1.0 milligrams	0. 5	3:3	2:3	0:3
1.5 milligrams	0. 75	2:3	2:3	1:3
3.0 milligrams	1. 5	3:3	[2] 1:1	0:3

[1] Control. [2] 2 died.

Another experiment with channel catfish (*Ictalurus punctatus*) indicated (so far as the effectiveness or the quantity of the pituitary used for injection is concerned) that glycerine extract was less effective than aqueous suspensions since only 1 female in 19 spawned. However, we feel that such a conclusion is unwarranted on the basis of this one experiment. The fish in the experiment had been starved for oxygen while being transported 2 days before use and had been both temperature-shocked and briefly oxygen-starved on the day the experiment began. After the end of the glycerine-extract experiments, nine fish which had received the glycerine injections were spawned by the injection of other gonadotropins (fish pituitary or human chorionic gonadotropin), but one might postulate that they had recovered from the shock, since the time of final spawning was several days later.

Crystalline penicillin at the rate of 10,000 units per injection was found to be an inexpensive and effective measure to combat inflammatory lesions in the body cavity due to irritation from the foreign proteins.

Although thousands of goldfish and the majority of experimental fish used in these studies usually spawned or ovulated after a single injection of pituitary materials without penicillin, it appeared later that in such fishes as the channel and flathead catfish penicillin acted to minimize the inflammation which resulted from the multiple injections of pituitary materials necessary to spawn these species. Our opinion was based on the difference between results we obtained before the routine use of penicillin and those we obtained at a later time with its use. No good, controlled experiments were conducted. In the original experiment that heralded this practice, goldfish received from 5,000 to 100,000 units of crystalline penicillin per dose for five injections over a 9-day period. Ground pituitaries were added to the injection on the 9th day, and the fish spawned on the 10th day in the manner expected. Now we recommend the injection of penicillin for all species as a precautionary measure. The penicillin is dissolved in distilled water, which is then added to the ground pituitaries. Dr. S. F. Snieszko recently suggested that chloramphenicol might be more effective for this purpose since it might also prevent gram negative bacterial infections, whereas penicillin is active against gram positive bacteria which do not often infect fish. The suggested dosage was 5 milligrams per 100 grams of body weight of the recipient.

Determination of active pituitaries

In our studies of inducing fish to spawn, it was desirable to learn the activity and relative potency of pituitaries collected throughout the year from various species of fish and from various types of individuals within the species, especially in regard to sex, maturity, and size. For these tests, bioassay was the only available means of evaluation. A test fish was needed, and the factors that determined its selection were as follows: (1) It should be readily available and easily kept in the laboratory. (2) It should respond predictably and uniformly to the injection of pituitary materials. (3) The nature of its response should lend itself to an objective rather than a subjective evaluation; for example, the ovulation and extrusion of eggs was used rather than visible increases in the weight of the gonad of the living fish. (4) Preferably, it should be an oviparous species of fish which spawns intermittently throughout the year.

There are several fishes that fulfill these requirements, and the selection of a test fish may vary with the prevailing circumstances. We have used female goldfish extensively during the normal spawning season (March-June) since they are more responsive at this time, and tests can be conducted with greater ease. Zebra fish (*Brachydanio rerio*), despite their small size, are good test fish because gravid females can be obtained anytime during the year; and since they respond to low dosages their use results in economy of pituitary materials. When female fish are employed, a positive test is obtained when the injection of pituitary material induces ovulation or precipitation of the spawning act.

We have also used male green sunfish (*Lepomis cyanellus*), carp (*Cyprinus carpio*), and goldfish (*Carassius auratus*) when ripe female fish were not available. Generally, these tests were not during the spawning season. Milt can be readily stripped from most males injected with pituitary material. The amount of the spermatic fluid will increase and will be less viscous. The response is particularly evident in out-of-season fish of species in which spermatogenesis is well advanced by late summer or early fall. For example, in midwinter the semen of carp (in which the spermatozoa are normally closely packed in the viscous seminal plasma) becomes very fluid and will actually drain from the fish about 20 hours after injection (Sneed and Clemens, 1956). A male which yielded about 0.1 milliliter of milt before injection produced about 10 milliliters after receiving a pituitary injection.

A similar response has been observed in the white crappie (*Pomoxis annularis*), white bass (*Roccus chrysops*), largemouth bass (*Micropterus salmoides*), goldfish (*Carassius auratus*), and golden shiner (*Notemigonus crysoleucos*). The increase of the fluidity of the milt after injection of pituitary has been observed in several other species of fish (Ihering and de Azevedo, 1934). Ihering (1935) used the length of time that the spermatozoa remained active when put into water as a measure of their maturity, and reported that pituitary injections into the donor male increased longevity of the sperm. We have not studied this effect, although we have observed changes in the osmotic pressure of the seminal plasma in carp which corresponded to the changes of fluidity (Sneed and Clemens, 1956). We feel that here is a potential method of measuring the relative activity of gonadotropins—the strength of the gonadotropin being equal to the difference in osmotic pressure of the seminal plasma before and after injection. For instance, carp sperm was immobilized with 85-percent Ringer solution (15 percent less water) while a 75-percent solution (more concentrated salts and higher osmotic pressure) was necessary for the inactivation of the sperm 15 hours after the fish had received one carp pituitary.

As the spawning season approached and the fluidity of the milt normally increased, the osmotic pressure of the seminal plasma increased—at least it required a solution of a higher salt concentration than it did earlier to maintain the sperm in an immotile state. Male zebra fish have not been used in this way, because the increase of milt is difficult to observe with the unaided eye. The morphology of the testes of channel catfish or of other ictalurids does not permit the use of hand-stripping methods; therefore the male of these species is not a potential test fish.

When the kind of fish and the type of test to be conducted has been determined, the individuals for the experiment must be selected. The selection of fish is discussed in the second part of this report.

Determination of dosages

When the experimental fish have been properly selected, suitable spawning facilities provided, and pituitaries of known activity secured, the determination of dosages may require a great deal of experimentation if the recipient species has never been spawned before. The major problems involved are: (1) How many milligrams of acetone-dried pituitary are required to precipitate spawning? (2) How many injections are required? (3) What is the optimum time interval between each injection?

If the species has not been spawned before, lots of three or more fish should be injected each 24 hours with a fairly wide range of pituitary dosages until spawning occurs. In our experiences only the females need to be injected during the spawning season. If there is any reason to doubt whether the male will spawn, perhaps he should be injected; however, the necessity for such injections has not been positively established for any species with which we have worked.

If the species has been spawned previously, the above questions have been partially answered. It is then a matter of matching the dose to the physiological condition of the recipients by administering doses in a graded series from above to below those recommended in this paper or those in other publications for other species of fish.

These pilot experiments will indicate the lowest dosage that will spawn the maximum number of fish with the minimum number of injections. Such a dosage should be determined for each lot of fish. It usually saves time and labor to administer doses slightly above the minimum required for spawning, rather than to risk failure with subminimal doses. Once a threshold dosage has been reached, no matter how much the dosage is increased, the results do not seem to change (table 3).

Phylogenetic specificity

One of the first questions to arise in the artificial spawning of any species of fish is what kind of pituitaries should be used for injection. In our experiments the goldfish has responded to injections of pituitaries from the following 15 species of fish representing seven families: Goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), bigmouth buffalo (*Ictiobus cyprinellus*), smallmouth buffalo (*I. bubalus*), black buffalo (*I. niger*), blue sucker (*Cycleptus elongatus*), gizzard shad (*Dorosoma cepedianum*), channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), flathead catfish (*Pylodictis olivaris*), black bullhead (*Ictalurus melas*), spotted gar (*Lepisosteus productus*), and extracted chum salmon (*Oncorhynchus keta*) pituitary (table 4). Low activity was obtained from the pituitaries of freshwater drum (*Aplodinotus grunniens*) and river carpsucker (*Carpiodes carpio*).

A series of experiments was conducted to determine whether such factors as the method of preservation of the pituitaries, the place of collection, the size of the donor fish, the amount of light to which the fish had been exposed, inhibiting materials in the pituitary, and the dosage level are responsible for the low activity of carpsucker pituitaries. Acetone-dried and lypholyzed pituitaries, pituitaries from fish from Lake Texoma and Fort Gibson Reservoir, pituitaries collected from December to April, and pituitaries from fish kept in the light and in the dark for a period of 3 weeks, all gave negative results when injected into goldfish that were known to be responsive to injections of a similar size of pituitaries from other kinds of fish.

TABLE 3.—*Response of different lots of goldfish* (Carassius auratus) *to pituitary dosages*

Dosage	Number injected	Number ovulated
Buffalo (*Ictiobus* spp.) pituitaries, Feb. 19, 1957, at 72° F.:		
0	2	0
⅕ pituitary	4	1
½ pituitary	4	3
1 pituitary	4	3
Buffalo (*Ictiobus* spp.) pituitaries, March 6, 1957, at 72° F.:		
0	4	0
0.2 milligram	4	1
0.3 milligram	4	2
0.9 milligram	3	0
1.7 milligrams	4	3
3.3 milligrams	4	3
4.9 milligrams	4	3
6.3 milligrams	4	3
Carp (*Cyprinus carpio*) pituitaries, March 18, 1957, at 72° F.:		
0	4	0
0.5 milligram	16	4
1.0 milligram	16	9
1.5 milligrams	32	21
Carp (*Cyprinus carpio*) pituitaries, April 7, 1958, at 60° to 62° F.:		
0	3	0
0.2 milligram	3	1
0.5 milligram	3	3
1.0 milligram	3	3
1.5 milligrams	3	3
3.0 milligrams	3	3

Carpsucker pituitaries are relatively small. The average weight of the thousands we collected and freeze-dried was 1 milligram, and these were collected from fish 10 to 15 inches long. When large dosages of either aqueous suspensions or acid-acetone extracts were injected, some activity was observed. Only 25 percent successful ovulations were produced in one experiment in which 3, 6, and 9 milligrams (4 to 10 glands) of carpsucker pituitary were injected in each fish of three groups of four goldfish. Another experiment in which acetone-dried pituitaries from carpsuckers were injected whole and without water by use of a pellet injector indicated that extremely high dosages were required to ovulate female goldfish. Four females received injections at each dosage level. No female ovulated at dosages of 1 milligram (1 gland); one ovulated with a dosage of 5.1 milligrams (5 glands), one with 10.2 milligrams (10 glands), two with 23

TABLE 4.—*Responses of 12 species of fish to pituitaries from donors of various taxonomic relationships*

Recipient species	Donor species	Intraspecific	Interspecific	Intrageneric	Intergeneric	Intrafamilial	Interfamilial	Intraordinal	Interordinal	Remarks
Goldfish (*Carassius auratus*)	Goldfish (*Carassius auratus*)	X	----	X	----	X	----	X	----	
Do	Carp (*Cyprinus carpio*	----	X	----	X	X	----	X	----	Responded to both acetone-dried pituitaries and acid-acetone extract (Robertson and Rinfret, 1957).
Do	Bigmouth buffalo (*Ictiobus cyprinellus*)	----	X	----	X	----	X	X	----	
Do	Spotted gar (*Lepisosteus productus*)	----	X	----	X	----	X	----	X	
Do	Flathead catfish (*Pylodictis olivaris*)	----	X	----	X	----	X	X	----	
Do	Channel catfish (*Ictalurus punctatus*)	----	X	----	X	----	X	X	----	
Do	Blue sucker (*Cycleptus elongatus*).	----	X	----	X	----	X	X	----	
Do	Gizzard shad (*Dorosoma cepedianum*).	----	X	----	X	----	X	----	X	
Do	Black bullhead (*Ictalurus melas*).	----	X	----	X	----	X	X	----	
Do	Blue catfish (*Ictalurus furcatus*).	----	X	----	X	----	X	X	----	
Do	Smallmouth buffalo (*Ictiobus bubalus*).	----	X	----	X	----	X	X	----	
Do	Black buffalo (*Ictiobus niger*)	----	X	----	X	----	X	X	----	
Do	Chum salmon (*Oncorhynchus keta*).	----	X	----	X	----	X	----	X	Cholesterol pellets of Robertson-Rinfret extractions; furnished by Roger Burrows.
Do	Freshwater drum (*Aplodinotus grunniens*).	----	----	----	----	----	----	----	----	May have been too low in activity for response at dosages administered.
Do	River carpsucker (*Carpiodes carpio*).	X	----	----	----	----	----	----	----	Some activity at higher dosage levels; positive with Robertson-Rinfret extractions.
Carp (*Cyprinus carpio*)	Carp (*Cyprinus carpio*)	X	----	X	----	X	----	----	----	Acetone-dried pituitaries, extracts, and fresh pituitaries.
Do	Buffalo (*Ictiobus* sp.)	----	----	----	----	----	----	----	----	
Do	Channel catfish (Ictalurus *punctatus*).	----	----	----	----	----	----	----	----	
Do	Goldfish (*Carassius auratus*)	----	----	----	----	----	----	----	----	
Golden shiner (*Notemigonus crysoleucas*).	Carp (*Cyprinus carpio*)	----	X	----	X	X	----	X	----	
White crappie (*Pomoxis annularis*).	Carp (*Cyprinus carpio*)	----	X	----	X	----	X	----	X	
Do	Chum salmon (*Oncorhynchus keta*).	----	X	----	X	----	X	----	X	Cholesterol pellets containing Robertson-Rinfret extract.
White bass (*Roccus chrysops*).	Carp (*Cyprinus carpio*)	----	X	----	X	----	X	----	X	
Zebra fish (*Brachydanio rerio*).	Buffalo (*Ictiobus* sp.)	----	X	----	X	----	X	----	----	
Do	River carpsucker (*Carpiodes carpio*).	----	X	----	X	----	X	----	----	Dosage level high.
Do	Carp (*Cyprinus carpio*)	----	X	----	X	----	X	----	----	
Channel catfish (*Ictalurus punctatus*).	Channel catfish (*Ictalurus punctatus*).	----	----	X	----	X	----	X	----	
Do	Carp (*Cyprinus carpio*)	----	X	----	X	----	X	X	----	
Do	Freshwater drum (*Aplodinotus grunniens*).	----	----	----	----	----	----	----	----	
Do	River carpsucker (*Carpiodes carpio*).	----	----	----	----	----	----	----	----	
Do	Buffalo (*Ictiobus* sp.)	----	X	----	X	----	X	X	----	
Do	Gar (*Lepisosteus* sp.)	----	X	----	X	----	X	----	X	
Do	Flathead catfish (*Pylodictis olivaris*).	----	X	----	X	X	----	X	----	
Rock bass (*Ambloplites rupestris*).	Buffalo (*Icitiobus* sp.)	----	X	----	X	----	X	----	X	
Emerald shiner (*Notropis atherinoides*).	Carp (*Cyprinus carpio*)	----	X	----	X	X	----	X	----	
Mimic shiner (*Notropis volucellus*).	Buffalo (*Ictiobus* sp.)	----	X	----	X	----	X	X	----	
Redfin shiner (*Notropis umbratilis*).	Buffalo (*Ictiobus* sp.)	----	X	----	X	----	X	X	----	
Flathead catfish (*Pylodictis olivaris*).	Flathead catfish (*Pylodictis olivaris*).	X	----	X	----	X	----	X	----	
Do	Buffalo (*Ictiobus* sp.)	----	X	----	X	----	X	X	----	
Do	Carp (*Cyprinus carpio*)	----	X	----	X	----	X	X	----	

milligrams (20 glands), four with 36.2 to 46.6 milligrams (40 glands), two with 54.7 to 70.2 milligrams (60 glands), and three with 78.2 to 98.1 milligrams (80 glands).

In almost all of the negative instances when 9 or more milligrams were injected, blood exuded from the oviduct when hand-stripping was attempted. This response suggested an overdose for those fish; but since the eggs in the fish that did ovulate appeared normal and no blood was seen, it cannot be implied that the amount of pituitary injected in the instances of negative response constituted a pharmacological overdose, but rather a physiological one.

Several species of fish were spawned with whatever kind of pituitaries were available (table 4). Phylogenetic specificity does not appear to be significant in fish-cultural practices (Pickford and Atz, 1957), and our experience bears out this viewpoint. Twenty-nine interspecific and intergeneric injections and 25 interfamilial injections were successful. Only seven heteroplastic injections failed to induce ovulation or exhibited low activity. Four of the seven failures involved the use of carpsucker and drum pituitaries at regular dosage levels, while the other three were unsuccessful attempts to spawn carp with heteroplastic pituitaries. However, carp were successfully spawned by other workers who used heteroplastic implants (see Pickford and Atz, 1957).

Kazanskii (reviewed by Pickford and Atz, 1957) implied that the carp, *Cyprinus carpio*, was in effect a universal donor. In our work, carp pituitaries successfully spawned nine species of fish. There may be other species of donor fish with equally active pituitaries, for instance, buffalo. However, carp pituitaries are usually more available, and their more extensive use has ed workers to regard them as superior. Pickford and Atz (1957) state that the work with fish provides clear indications, but no proof, of specificity. Our present studies indicate that there was little specificity between families and lower taxonomic categories. At least, the differences were so slight that they have no practical importance in fish-cultural techniques. However, an explanation is certainly needed for the lower activity of carpsucker and drum pituitaries. It may be that the carpsucker pituitaries were taken largely from immature fish. Drum pituitaries have not been tried in enough experiments to make a definite conclusion, although we have used them a dozen times or more.

Gonadotropic activity in relation to season

It is important to be relatively certain that the pituitaries collected for the purpose of injection are active, as injections with inactive materials would waste valuable time and perhaps fish. We attempted to devise experiments that would determine the seasonal activity of the pituitaries of several species of fishes, but our supply of pituitaries was not always sufficiently large to provide materials for the basic experiments of inducing spawning in channel catfish as well as for comparative tests of materials collected in different months. The limited significant data on seasonal activity that we obtained are presented in table 5. These data represent successful attempts to induce ovulation or spawning of several species and indicate that the pituitaries were active in each of the months listed. We have no data which indicate that the pituitaries of any species are inactive during any month in which we made collections. The pituitaries of the carp proved to be active in at least 9 months of the year (we did not test carp pituitaries collected in May, August, or September); those of buffalo (*Ictiobus* spp.) were active in 8 months, including May and August (table 5). No seasonal difference in pituitary activity was discernible from the injections which

TABLE 5.—*Activity of pituitaries collected in different months from eight donor species*

[X indicates that spawning was induced when the pituitaries were injected in any of several test species]

Species	Donor fish collected in—											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Carp (*Cyprinus carpio*)	X	X	X	X	-------	X	X	-------	-------	X	X	X
Buffalo (*Ictiobus* spp.)	X	X	-------	-------	X	-------	X	X	-------	X	X	X
River carpsucker [1] (*Carpiodes carpio*)	X	X	X	X	-------	-------	-------	-------	-------	-------	-------	X
Flathead catfish (*Pylodictis olivaris*)	X	-------	-------	-------	-------	X	X	-------	-------	-------	-------	X
Gar (*Lepisosteus* spp.)	-------	-------	-------	-------	-------	-------	X	-------	-------	-------	X	-------
Goldfish (*Carassius auratus*)	-------	-------	X	X	-------	-------	-------	-------	-------	-------	-------	-------
Channel catfish (*Ictalurus punctatus*)	-------	X	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Blue sucker (*Cycleptus elongatus*)	-------	-------	-------	-------	-------	X	-------	-------	-------	-------	-------	-------

[1] Pituitaries of this species only slightly active.

we made to induce spawning. This fact does not necessarily indicate that seasonal differences do not exist. We believe that our experiments were not sufficiently sensitive to indicate variability in pituitary activity from month to month that could not be explained equally well on the basis of inconsistencies in the bioassay method and/or the individual physiological variability of the recipient species.

On the basis of the information now available on fish gonadotropins, the biological assumption may be made that there is a seasonal difference in the amount of hormones in the blood or in the amount utilized by the target organ. This assumption does not necessarily include a difference in the concentration of gonadotropins in the pituitary itself. Gerbil'skii (reviewed by Pickford and Atz, 1957) generally concluded "that the presence—and therefore presumably the production—of fish gonadotropin is low only for a period of varying length following spawning." He believed that the gonadotropic content of pituitaries from fish that spawned in the spring remained relatively low into October. However, his data are not conclusive, and as we have pointed out many times, the bioassay method is inconsistent, and often the recipients may vary physiologically more than the pituitaries vary in gonadotropic content. Unlike Brazilian practices of collecting glands from only ripe or ripening fish, we recommend the collection of pituitaries from mature fish during any month of the year at the convenience of the worker.

Gonadotropic activity in relation to sex of donor

Pickford and Atz (1957) reviewed the literature on differences in gonadotropic content of the pituitary of males and females. They stated that although fish-culturists use the glands of either sex indiscriminately, they can no longer assume that the gonadotropic content of male and female pituitaries is the same. They cited the work of Barannikova (1949) which showed that pituitaries of male sevringa (*Acipenser stellatus*) when tested in *Misgurnus fossilis* were at times only half as potent as those from females. With the donors and recipients used in our work and in large-scale operations in fish culture, we have found nothing to be gained from separating the pituitaries of the two sexes. In practical application, when fully ripe fish are used, selected dosage levels are usually more than minimal, since there is no apparent harm to the fish or the spawn with moderate overdoses. Slight overdoses tend to offset individual differences in both the pituitaries and the recipient fish. Such differences in activity due to sex or other variables are further minimized by grinding together pituitaries of various collections and injecting known quantities of the ground mixture.

TABLE 6.—*Response of female goldfish* (Carassius auratus) *injected with whole or with parts of transected buffalo* (Ictiobus *sp.*) *pituitaries*

Kind of pituitary injection	Weight of pituitary injected (milligrams)	Number of injected fish	Number of ovulating fish	Part of pituitary injected	Weight of pituitary (milligrams) before transection	Number of injected fish	Number of ovulating fish
Whole	6.3	4	2	Anterior	1.6	4	3
Whole	4.9	4	3	Posterior		4	0
Whole	3.3	4	3	Anterior	1.5	4	3
Whole	1.7	4	3	Posterior		4	0
Whole	0.8	4	0	Anterior	3.1	4	[1] 2
Whole	0.4	4	2	Posterior		4	1
Whole	0.3	4	1				
Control	0	4	0				

[1] The negative fish in this group were considered poorly developed at the time the experiment began.

Site of gonadotropic activity in the pituitary

In order to determine which part of the pituitary contained the active gonadotropins, an experiment was designed which (1) determined a dosage of whole buffalo pituitary (about 1.7 milligrams per fish, table 6) necessary to induce ovulation in at least three of four goldfish (this information was necessary to assure the use of pituitaries of adequate size in the second part of the experiment) and (2) tested the gonadotropic activity of the anterior and posterior parts of three pituitaries of approximately similar size or larger by injecting each part into separate groups of four goldfish (table 6). Posterior lobe here refers to the meta-adenohypophysis, as suggested by Pickford and Atz (1957); anterior lobe refers to the remainder of the pituitary.

In evaluating the response of the injected fish approximately 15 hours after injection, only those fish that readily responded to hand-stripping were counted as positive. About 69 percent of the females responded to injections of ground whole pituitaries when the dosage was 1.7 milligrams or more; 71 percent of the females responded to injections of the anterior lobes of pituitaries weighing 1.5 milligrams or more; no ovulation occurred in the eight fish which received the posterior part of the pituitary. These results of the effect of buffalo pituitaries on goldfish confirmed reports that the source of gonadotropic activity is found in the anterior lobes of the pituitary, and that the posterior lobe contains no active gonadotropins. When the dosage levels were doubled, results were as expected—positive results with the anterior portion and some activity with the posterior portion. Eggs were easily hand-stripped from one female injected with posterior lobe. These results were probably the outcome of the method of dissection: pieces of the anterior lobe were left on the posterior lobe so that enough anterior lobe material had accumulated to be active in the higher injection. Other workers (Kazanskii and Persov, Barannikova, see Pickford and Atz, 1957, for review) transected pituitaries and demonstrated that the anterior portion is the site of gonadotropic activity.

Relation between pituitary size and gonadotropic activity

The gonadotropic activity of different sizes of pituitaries (acetone dried) was indicated by the percentage of induced ovulation of goldfish injected with standard amounts (1.5, 1.0, and 0.5 mg.) of pituitaries of varying sizes, e.g., eight groups of carp pituitaries varying in size from one to eight per 10 milligrams (combined weight) and five groups of buffalo

TABLE 7.—*Gonadotropic activity of various sizes of carp and buffalo pituitaries as indicated by induced ovulation of injected goldfish*

[Data based on fish rated "good"]

CARP PITUITARIES

Number of carp pituitaries per 10 milligrams	Dosage 1.5 milligrams		Dosage 1.0 milligrams		Dosage 0.5 milligrams	
	Number injected	Percent of ovulation	Number injected	Percent of ovulation	Number injected	Percent of ovulation
1	2	100	3	66.6	2	50
2	4	100				
3	3	100	2	0	1	100
4	4	100				
5	2	100				
6	1	100	2	100	1	0
7	3	100				
8	1	0	3	100	1	0

BUFFALO PITUITARIES

Number of buffalo pituitaries per 6.5 milligrams	Dosage 1.5 milligrams		Dosage 1.1 milligrams		Dosage 0.75 milligrams		Dosage 0.37 milligrams	
	Number injected	Percent of ovulation	Number injected	Percent of ovulation	Number injected	Percent of ovulation	Number injected	Percent of ovulation
1	2	50	1	100	2	50	2	0
2	3	66.6	1	0	4	100	2	100
3	1	100	4	75	2	50	1	100
5	2	50						
6	3	100						

pituitaries varying from one to six per 6.5 milligrams (table 7).

The recipient fish for these experiments were not selected, but were arbitrarily assigned to aquariums and rated as good, fair, and poor; thus the results reflect the condition of these fish as well as the activity of the pituitaries of the donors. The ratings were made before the experiment, and we used only the data obtained from fish rated "good".

A fish rated "good" was one we believed to have a good possibility of ovulating or spawning, doubtful fish were rated "fair," and "poor" fish in all probability would not ovulate or spawn. At the dosage of 1.5 milligrams per fish all of these estimates of condition were above 80 percent correct—94.2 percent correct for the "good" ratings, 80 percent for "fair," and 100 percent for "poor."

If only the fish rated "good" were considered at the standard dosage level (1.5 milligrams) for any size of pituitary, then the size differences were not significant provided the total weight of pituitary was the same (table 7). Therefore, the activity per unit weight did not vary with the size of carp pituitaries weighing from 1.0 to 10 milligrams, the gonadotropic activity being directly proportional to the weight of the pituitary. If there was a difference in hormonal activity, it was less than the differences in the response of various individual fish.

In practice then, variations in size of pituitaries may account for differences in effectiveness of the injections if pituitaries are injected without weighing. It is suggested that pituitaries used for injections be combined, finely ground, thoroughly mixed, and weighed for injection to provide more uniform results.

Relation of pituitary weight to fish length and weight

We have demonstrated that the size of the pituitary in mature fish is a general indicator of its potential gonadotropic activity. We also endeavored to determine how the size of the pituitary varied with the size of fish. Pituitaries were removed and weighed immediately after the fish were killed. Average pituitary weight for each length group of gravid goldfish was plotted against that group to provide the line of figure 1. It clearly demonstrates a straight line relationship. The regression equation for the grouped data is $Y=1.30-6.31$ and the coefficient of correlation is 0.892. However, there is considerable variation in pituitary size as related to length of fish, as indicated in the range of pituitary weights for each group of fish lengths (table 8). A small portion of this variation may be due to the fact that different amounts of the stalk and membranes were removed and weighed with the pituitary and that slightly different amounts of moisture remained on each pituitary after blotting.

A similar straight-line relation between fish length and pituitary weight is shown for the male and female channel catfish (fig. 2, based on data in table 9). The regression formula for males is $Y=1.55x-15.55$ and for females it is $Y=3.40x-44.44$. The coefficient of correlation for males is 0.887, and for females it is 0.817. When pituitary weight was plotted against fish weight (fig. 3) the regression equation was $Y=3.56x+4.12$ for males and $Y=8.14x-3.31$ for females. The correlation for males is 0.911 and for females it is 0.952. Thus, there is a slightly higher correlation between pituitary weight and fish weight than there is between pituitary weight and fish length.

TABLE 8.—*Relation of pituitary weight (wet) to fish length of gravid goldfish*

Total length of fish	Range of weights (milligrams)	Average weight (milligrams)	Number of pituitaries
5.0 inches	0.8	0.8	1
5.5 inches	1.0- 1.2	1.1	2
6.0 inches	0.7- 3.6	1.4	8
6.5 inches	1.5- 3.2	2.1	4
7.0 inches	0.8- 4.2	2.1	10
7.5 inches	2.0- 3.8	3.1	3
8.0 inches	0.9- 6.0	3.8	7
8.5 inches	1.2-10.2	6.0	5
9.0 inches	----------	----------	----------
9.5 inches	6.1	6.1	1

TABLE 9.—*Relation of pituitary weight to fish length of channel catfish*

[Only one fish was used in each length listed]

Length of fish	Weight of fish (pounds)	Wet weight of pituitary (milligrams)
Male channel catfish:		
15.4 inches	1.6	11.5
15.5 inches	1.5	11.0
16.2 inches	1.4	8.5
16.7 inches	1.7	12.0
17.8 inches	1.9	12.0
18.1 inches	2.4	8.5
18.1 inches	2.4	9.0
18.5 inches	2.5	15.0
20.8 inches	2.8	14.0
20.8 inches	4.0	15.0
22.4 inches	3.4	18.0
24.0 inches	5.6	25.0
25.0 inches	5.4	25.0
Gravid female channel catfish:		
13.8 inches	.9	4.5
14.5 inches	1.3	6.1
15.2 inches	1.2	6.3
16.0 inches	1.4	8.2
16.9 inches	1.6	13.3
17.6 inches	2.2	11.5
17.6 inches	2.2	12.4
19.3 inches	3.4	26.0

The ratio of the weights of fresh to the acetone-dried pituitaries for 26 female goldfish was 3.13:1 (24 percent of the wet weight). The ratio of the average wet weight of the pituitaries to the average weight of the gravid female goldfish was 1 milligram of pituitary to 34.9 grams of fish, or 13 milligrams per pound of fish. This ratio ranged from 5.7:1 to 26.9:1. The ratio of the wet weight of the pituitary in milligrams to the weight of female channel catfish in pounds was 6.2:1; for males 5.4:1 (table 9).

Even though the relative size of the pituitary varies phylogenetically, the fact does not appear to be of any practical significance to fish-cultural practices. We have used standard dosages based on milligrams of pituitary per pound of body weight of the recipient, regardless of the size, sex, and species of the donor. This practice may have to be changed for the pituitaries of certain species, e.g., river carpsucker and drum, as more information is gained, since our data indicate that the pituitaries of these species are low in activity.

Pickford and Atz (1957) reviewed the literature concerning the percentage of

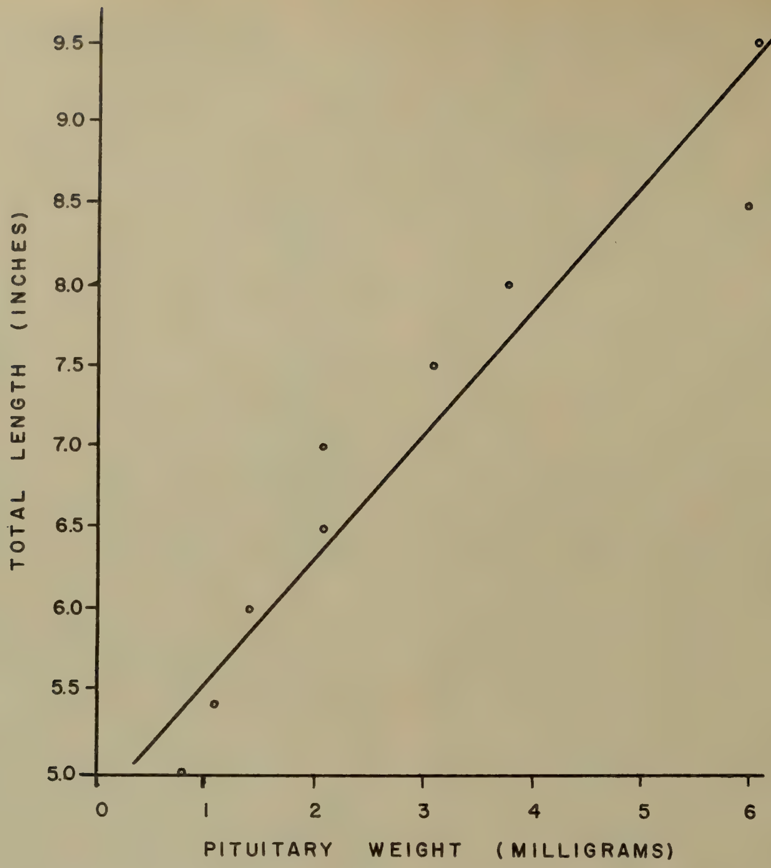

FIGURE 1.—Relation between pituitary weight and total length of gravid female goldfish.

weight loss in drying pituitaries in acetone and found that the defatted, dry weight represents 13 to 17 percent of the wet weight of the pituitaries of four different teleosts, which, it might be noted, belong to three different orders, two of the species being freshwater, one anadromous, and one marine.

Latent period

The time of response from injection to ovulation (the latent period), provided that it is rather constant, has practical significance in fish culture. Knowledge of this latent period permits more efficient hatchery operation insofar as the use of fish, men, and equipment are concerned. We have noticed that goldfish carefully selected for their apparent advanced spawning condition and injected in the afternoon invariably spawn early the next morning if the water temperature is about 70° F. At lower temperatures, a somewhat longer period is required.

These observations raised the question of whether the fish respond in a given interval of time or whether the exogeneous gonadotropins ready the fish physiologically and they spawn early the next morning when light and other environmental conditions are favorable for normal spawning. The following experiment was designed to answer this question.

Four female goldfish were each injected with 2.6 milligrams of acetone-dried carp pituitary collected in December. At 2-hour intervals, other groups, each of four fish, were similarly injected. This proce-

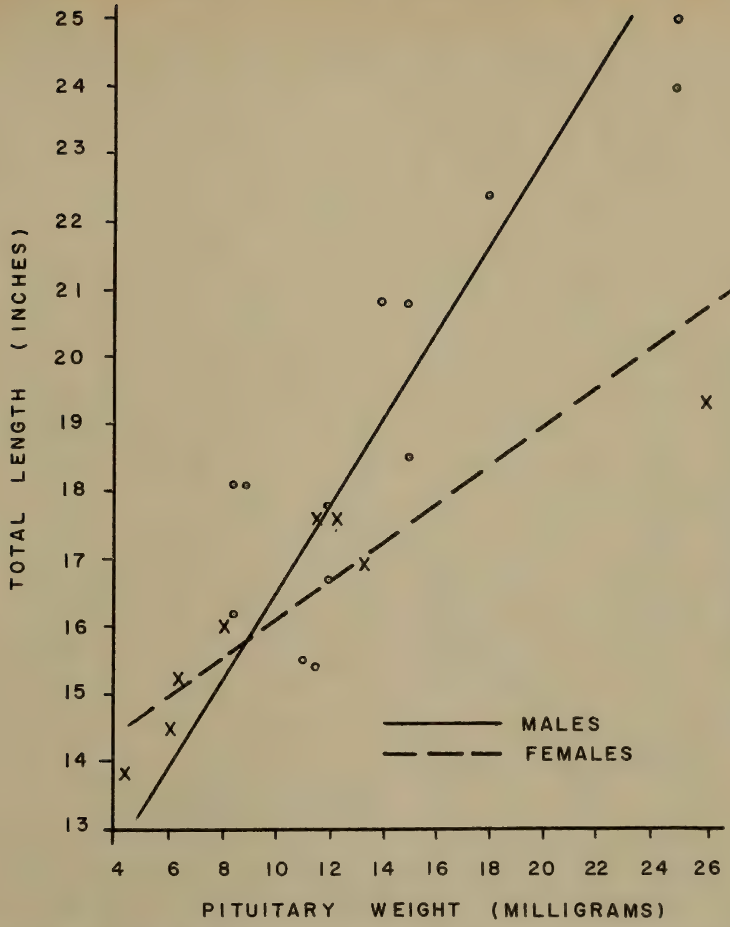

FIGURE 2.—Relation between pituitary weight and total length of male and female channel catfish.

dure was continued for a 12-hour period. All injected fish were examined every 2 hours. Presumably, if goldfish responded at a given time after injection, the fish which we first injected would spawn after that interval of time, and the others would spawn each 2 hours during the next 12 hours. If they spawned at a particular time of day, there would be no 2-hour pattern; the fish would spawn only in the morning.

No eggs could be hand-stripped from the selected fish prior to the pituitary injection. On subsequent attempts, if a few eggs could be stripped, the condition was designated as "early ovulation" (table 10). In other instances, hand-stripping released a considerable number of eggs which were closer to the color and texture of ripe eggs; this condition was designated as "incomplete ovulation". Only when the eggs could be stripped from the fish with slight pressure was the fish considered to be fully ripe and its condition rated as "complete ovulation". Admittedly the classification is a matter of judgment, but the four people who worked in the laboratory readily agreed upon the condition of the fish and eggs in all cases.

Of the 28 fish injected throughout the course of the experiment, all fish that ovulated did so within 12 to 18 hours after injection. One fish responded in 12 hours, seven in 14 hours, nine in 16 hours, and

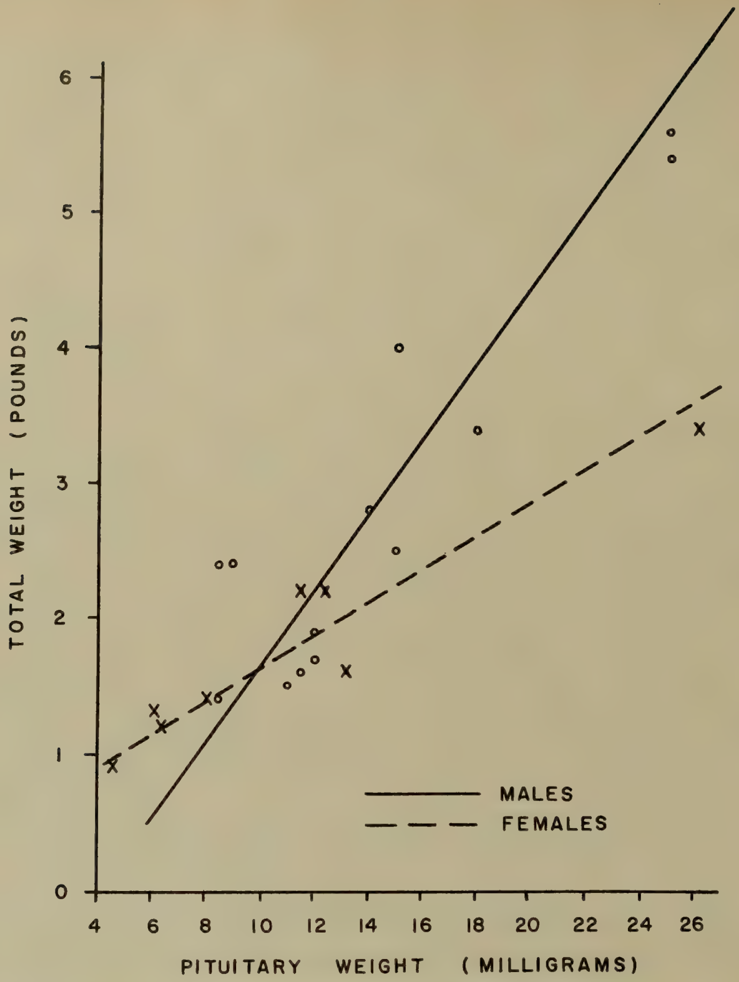

Figure 3.—Relation between pituitary weight and weight of male and female channel catfish.

two in 18 hours. Sixteen fish, about 90 percent, responded between 14 and 16 hours at a temperature of about 72° F. Nine fish did not ovulate.

The fact that 90 percent of the females responded to hormone injections after 14 to 16 hours is significant to the fish-culturist. For example, if goldfish are injected during the late afternoon, spawning should occur during the early morning hours when the sun is coming up and when the goldfish normally spawn. However, if one desires spawn during other hours, fish can be injected at times calculated to give spawn at any hour which might be advantageous to the culturist.

It is not particularly significant that 9 of 28 females did not ovulate, since the fish employed were not selected to be completely ripe. The pituitary method of spawning as used in this experiment was not intended to ripen fish, but merely to precipitate ovulation in ripe fish. The spawning of unripe fish is another problem and involves different techniques.

The length of the latent period of goldfish varies with temperature. If the fish were ripe enough to respond at all, the latent period increased from 12 to 25 hours as the temperature decreased from 85° to 52° F., the range of temperatures at which goldfish were spawned in our ex-

TABLE 10.—*Latent period for female goldfish injected with 2.6 milligrams of carp pituitaries at 72° F.*

Size	Time of injection	Number of hours from injection of pituitaries to spawning		
		Early ovulation	Incomplete ovulation	Complete ovulation
7.25 inches	0815	------------	14	16
8.5 inches	0815	------------	------------	16
8.0 inches	0815	12	14	------------
7.25 inches	0815	12	14	18
8.0 inches	1015	------------	------------	16
7.75 inches	1015	10	------------	18
7.5 inches	1015	10	24	------------
7.25 inches	1015	14	------------	16
8.75 inches	1215	------------	------------	16
8.5 inches	1215	------------	------------	14
6.75 inches	1215	12	------------	14
7.5 inches	1215	------------	------------	16
7.25 inches	1415	------------	------------	16
7.0 inches	1415	------------	20	------------
7.25 inches	1415	4	8	------------
6.75 inches	1415	10	18	------------
8.25 inches	1615	2	------------	14
7.75 inches	1615	2	18	------------
9.0 inches	1615	------------	------------	14
7.5 inches	1615	------------	18	------------
7.5 inches	1815	------------	------------	16
7.0 inches	1815	8	------------	16
8.0 inches	1815	------------	------------	12
6.25 inches	1815	2	16	------------
8.0 inches	2015	------------	------------	------------
8.5 inches	2015	------------	------------	14
7.5 inches	2015	------------	------------	14
6.5 inches	2015	------------	------------	14

periments. If certain goldfish require considerable ripening and more than a single standard injection is necessary to precipitate spawning, the time of response from the first injection is probably more contingent upon the amount of maturation required than on the dosage level, if the level is equal to or above the standard dose determined for ripe females in the same lot of fish. This statement is made in light of the fact that the strength of the injection does not seem important to the induction of spawning or to the latent period of ripe goldfish once a certain level or threshold is reached. Since gonadotropins are probably excreted or inactivated, the excess quantities therefore would not stimulate ripening in direct proportion to the dosage level, but would be somewhat less than proportional. The fact that the length of the latent period was fairly constant probably means that the threshold dosage existed, and the injected fish as a group were of similar physiological condition. One thing that should be noted here is that different groups of goldfish do have different quantitative requirements for a threshold dosage (table 3), which again emphasizes the importance of the spawning condition of the recipient and the experience of the fish-culturist in judging that condition.

Another point to be made in regard to the variable latent period (12 to 18 hours) is that after 18 hours no fish continued development to the "complete" ovulation stage, suggesting that the absorption of hormones from the 2.6 milligrams of pituitary was virtually complete after 18 hours. In another paper (Sneed and Clemens, 1958), our data on channel catfish suggest that the level of gonadotropins necessary to produce spawning was maintained more effectively by small dosages (every 8 hours) than by larger dosages less frequently given (24 hours or longer).

Effect of exogenous gonadotropins on fertility and development

Pickford and Atz (1957) stated in connection with their review of Russian literature that "there is ample evidence that improper application of the method of inducing ovulation by pituitary injection can yield inferior sex products." "Improper application" means too large a dose, and "inferior sex products" means infertile eggs or sperm, reduced viability, the incidence of monsters, and in the case of sturgeons, parthenogenetic development of the eggs. However, the Russian data are not clear cut, and Russians themselves argue the point.

No such effects have been detected in incubating and hatching the eggs, or in raising the fingerlings from over 200 channel catfish spawns obtained by pituitary injections. Some decrease in fertility (1 to 5 percent) was observed when the same male was used to spawn two or more females, and in the spawns from fish early or late in the season. Such instances of infertility can be accounted

TABLE 11.—*Fertility of eggs from carp and goldfish injected with acetone-dried pituitaries 15 to 24 hours before handstripping.*

Female	Male	Percent fertility	Remarks
Goldfish	Goldfish	99	Development followed to hatching.
Goldfish	Goldfish	91	Development followed to hatching.
Goldfish	Goldfish	92	Development followed to hatching; unfertilized eggs stored in plastic bag 1 hour before fertilization.
Goldfish	Carp	75	
Carp	Goldfish	97	Eggs stripped into Ringer's solution immediately before fertilization.
Carp	Goldfish	91	
Carp	Carp	97	
Carp	Carp	90	Sperm stored in Ringer's solution before use.
Goldfish	Carp	80	Unfertilized eggs stored in plastic bag in refrigerator 1 hour; sperm stored 4 hours in Ringer's solution.
Goldfish	Carp	80–85	Unfertilized eggs stored in refrigerator 1 hour.

for on the basis of incomplete development early in the season and natural deterioration of eggs late in the season. It was not uncommon for the eggs to be inferior in the first few ovipositions, and on occasion these were eaten by the spawning fish. The lowered fertility was never great enough to be of practical significance; and it was never attributed to hormone injections, except when fish were hormone-spawned before their normal spawning season, or late in the season.

Excellent fertility was demonstrated by handstripping carp and goldfish of both sexes, which were previously injected with acetone-dried pituitaries (table 11). Similar results were obtained when the injected fish were allowed to spawn on mats.

Exogenous gonadotropins can be used successfully to spawn fully developed females. Inferior results, as reported in the literature, probably involve extremely large doses of pituitaries, faulty techniques, or the use of unripe or delayed fish. From a fish-cultural standpoint, the understanding of why some fish are refractive to hormone injections is more important at the present than is an explanation of slightly harmful effects of gonadotropins on fertility and development.

USE OF PITUITARY MATERIALS

A review of the attempts to spawn fish by hormones reveals a number of techniques which produce varying degrees of success and failure. The reason that a proper and proved technique may produce negative results lies to some extent with the qualifications of the investigator who usually is either an endocrinologist who knows almost nothing about fish culture or a fish-culturist who is rather uninformed about endocrinology. For this reason, we regard only positive results in the reports of induced spawning experiments as truly significant. Sometimes even the positive results need to be regarded with caution since fish occasionally can be chemically or physically shocked into spawning.

The condition of the female fish at the time that induced spawning experiments are conducted is of utmost importance. The method presently used and discussed in this paper concerns spawning of ripe fish only. Within relatively narrow limits, fish can be hastened into spawning by pituitary injections. Likewise, fish slightly delayed in spawning, or refractive to spawning in their natural habitat, can be spawned by these methods. The manipulation of fish beyond these narrow limits is another problem.

The best method of selecting fish for advanced sexual development is to examine the larger fish in the hand; smaller (minnow-sized) fish can be examined in a quart jar. The assessment of the females' physiological receptivity to gonadotropins was based on a combination of the following characters: (1) Abdominal distention by the ovaries, preferably after the digestive tract has been emptied; (2) condition of the genital opening; (3) color; (4) degree of abdominal flaccidity; (5) behavior.

The assessment of the degree of sexual development of the male is not usually a major problem. During the spawning season, males of many species often show sexual dimorphism, and any male observed in breeding color was assumed to be ripe. If sexual dimorphism does not exist, and it is difficult to determine ripe males from unripe females and immature individuals, the fish of undetermined sex can be stocked at a ratio of two or more per gravid female in an effort to assure the presence of some mature males in the tank. These fish should be similar in size to the selected gravid females. In those species that pair, the behavior of the male frequently indicates whether or not he is a potential spawner. His positive or negative attitude toward the female or to other males often reflects his sexual development. A thorough knowledge of the natural spawning behavior and conditions for the particular species makes it much easier to select fish that can be induced to spawn. Both males and females are usually injected for handstripping procedures, but otherwise only the females receive gonadotropins. For species that require the more specific environments for spawning, such as the nest builders, it may be necessary to provide certain facilities such as vegetation, gravel, rocks, cans, or flowing water, since stimuli from the environment may also play an important role in the proper metabolism and activity of the injected hormones.

Other important factors of general technique to be considered include dosage and handling. It usually saves time and labor to give doses above the minimum required for spawning, rather than to administer a subminimal dose. The evidence indicates that once the spawning dose is reached greater amounts of pituitary do not change the results (table 3).

Our general technique varied with the size of the fish. Minnow-size fish (2 to 3 inches long) were usually anesthetized in 1:10,000 MS 222 and were held in the folds of a net. They were injected intraperitoneally with 0.1 to 0.2 milligrams of pituitaries in 0.1 to 0.2 milliliters of distilled water which contained 100 to 200 units of penicillin. If greater volumes were injected, the fluid was more likely to be lost from the body cavity after the needle was withdrawn. Also, greater volumes produced an edema and peritoneal inflammation, perhaps because the internal pressure was too great for the welfare of the fish, or the volume of fluid was too great for complete absorption. For these small fish, a needle size of 26 or smaller was used. For larger fish, needle sizes from 19 to 21 were satisfactory and did not often clog. Luer-lock hypodermic syringes, which have a locked needle, will prevent the loss of materials if the needle clogs. Large fish were not anesthetized. They were injected intraperitoneally with pituitary dosage of 2 milligrams or more per pound of body weight, with 1 milliliter or more of distilled water, and 10,000 units of penicillin if multiple injections were necessary.

Suitable facilities and proper spawning places (such as well-aerated running water, aquaria, troughs, pens, or ponds) must be provided, the type depending on the species to be spawned. If a question of water temperature arises, a temperature approximating that at which spawning occurs in nature is used. If there is danger of the eggs being eaten, some species (e.g., zebra fish) can be spawned over netting through which the eggs can settle and be separated from the parent fish. Other species (goldfish, carp, etc.) can be spawned on mats which are periodically moved to hatching ponds or troughs. The egg masses of the catfishes are easily moved to mechanical hatching facilities immediately after spawning is complete.

An account of our experience in spawning twelve species of fish is given in the following sections and is summarized in table 12. It may appear to the reader that in the following sections that not enough attention has been given control experiments. In those species (goldfish, carp, channel catfish and golden shiner) that were spawned on a large scale, control groups were usually not set up at this stage of our investigations, since many previous controlled experiments had indicated that these species did not often spawn in confined spaces (aquaria, holding troughs, etc.) without pituitary injections.

In the case of the zebra fish, too few controls were used to demonstrate a clear-cut difference between the response of injected and uninjected fish. Since this fish readily spawns in small containers, the effect of gonadotropins never will be as pronounced.

The information on the spawning of the remaining species should be considered as preliminary, since only a few fish have been spawned. When only a few fish are involved, negative controls have little meaning, since the fish are so individualistic in their physiological condition. Only random injections and random controls from a particular population, and then only if many individuals are used, give statistical significance to controlled experiments involving induced spawning in many species of fish. For such species as the channel catfish, which, when held captive in small aquariums, does not spawn unless it is injected, controls appeared superfluous after this fact was definitely established. Also, for all species with which we worked, control information was accumulated inasmuch as similar, uninjected fish were almost always maintained in the laboratory under practically identical conditions, although formal controls were not designated.

The response of a particular species of fish to pituitary injections varies with the gonadal development of the fish. Very gravid females respond much more readily than fish less well developed. In other words, it is easy to induce spawning during the regular spawning season, the fish often spawning with one injection; whereas fish injected several weeks before their regular spawning season may require several injections over a period of hours or days in order to precipitate the spawning act. Usually several injections must be used to spawn fish which have been delayed past their normal spawning period, but spawning cannot be obtained if degeneration of eggs has begun. Usually delayed fish are refractory, but quite often normal eggs can be procured if several low-level dosages are give. For example, we have spawned carp as early as February and as late as July 14 by use of the pituitary injection method, and Mr. Mayo Martin (personal communication) spawned carp in September in Arkansas. There are records in the literature of cyclic-spawning fish having been spawned during practically every season of the year (Hasler and Meyer, 1942; Kawamura and Otsuka, 1950; Hasler, Meyer, and Field, 1939; Ball and Bacon, 1954). Apparently, the major requirements for successful induced spawning are that fish contain well-developed eggs and that the injected pituitaries contain the components necessary to precipitate the spawning act or to produce ovulation. In many species (carp, goldfish, channel catfish, green sunfish), sperm is available in at least some males most months of the year (see also Pickford and Atz, 1957, p. 216).

Chronic injections of pituitary materials at dosage levels normally used to induce spawning in a particular species can be harmful to fish that contain small, immature eggs. For example, 1-year-old female largemouth bass (*Micropterus salmoides*) whose ovaries contained mostly immature, the amount of pituitary which tracted chum salmon pituitaries in choles-

TABLE 12.—*Summary of induced spawning experiments for twelve species of fish*

Species	Size of recipient	Number spawned	Month	Water temperature (°F.)	Donor species	Dosage	Number of milliliters	Number of injections	Facilities	Can be hand-stripped?
Goldfish (*Carassius auratus*).	3–12 inches	Thousands	Mar.–June	65	13 species	1 gland	1.0	1–2	Aquariums, troughs, ponds, mats.	Yes.
Carp (*Cyprinus carpio*)	1 lb.	Hundreds	Mar.–June	75 (65–85)	Carp	1 gland (1–5 mg).	1.0	1–2	Troughs, ponds, mats	Yes.
Golden shiner (*Notemigonus crysoleucas*.	3–5 inches	Hundreds	May	68	Carp	1/20–1 gland	0.2	1	Aquariums, ponds, mats	Yes.
Emerald shiner (*Notropis atherinoides*).	2½–3½ inches	1 to 3	July	76	Buffalo	1/10 gland	0.2	1	Aerated aquariums; fish over netting.	Not known.
Mimic shiner (*Notropis volucellus*).	2 inches	1 to 8	June	76	Carp	1/10 gland	0.2	1	Areated aquariums; fish over netting.	Not known.
Redfin shiner (*Notropis umbratilis*)	1½–2 inches	1 to 4	July	76	Buffalo	1/10 gland	0.2	1	Aerated aquariums; fish over netting.	Not known.
Channel catfish (*Ictalurus punctatus*).	0.7–8.2 pounds	200	May–June	70 (70–85)	5 species (see text).	2 mg./lb.	1.0	3	Aquariums, troughs, pens	No.
Flathead catfish (*Pylodictis olivaris*).	5.0 lb.	4	July	75	3 species (see text).	2–6 mg. per lb.	1.0	3–4	Aquariums, troughs	No.
White crappie (*Pomoxis annularis*).	10–12 inches	4	May	61	Carp, Saline extract.	4 mg	1.0	1–2	Troughs, mats	Yes.
Rock bass (*Ambloplites rupestris*).	5–7 inches	2	July	76	Buffalo	2 mg	1.0	2	Aquariums, gravel, running water.	Not known.
Zebra fish (*Brachydanio rerio*).	1–2 inches	Several	Dec.–May	80	3 species (see text).	0.6 mg	0.2	1	Aerated aquariums; fish over netting.	Yes.
White bass (*Roccus chrysops*).	9–12 inches	2	June	72	Carp	0.5 and 2 mg	1.0	2	Trough, mats	Yes.

terol pellets (prepared by Dr. Arthur Rinfret and supplied to us by R. E. Burrows). These fish became nervous after 5 or 6 days, began to lose mucus from the body, developed whitish spots, and jerked violently when handled. The muscles of the fish felt hard, as though the fish had been dehydrated. On the second day the fish gave off large quantities of urine when stripping tests for eggs were made, and would often flex into a rigid sigmoid curve when handled. Fish responded similarly to large amounts of fresh pituitary given frequently over a 24-hour period, or when large doses were administered daily for 6 or 7 days. We have noticed a few identical responses in other species including crappie, goldfish, carp, and zebra fish. There are several possible explanations for such phenomena:

1. When gonads are poorly developed or immature, the amount of pituitary which we injected disrupted metabolic processes of the fish, i.e., the injections constituted a pharmacological overdose.
2. The extract which we used and the raw pituitaries contained products which were toxic to the fish.
3. The fact that the fish appeared dehydrated and that the urine was increased suggests that hormones had a diuretic effect on the fish.

The toxicity of some pituitary materials to immature fish may possibly be due to ACTH, as suggested by Roger E. Burrows (correspondence).

Two results of our research suggest that the hormonal requirements of the two groups of fish are different quantitatively and/or qualitatively: (1) Sexually mature fish respond in an apparently normal fashion to dried, whole pituitary and to pituitary extracts; (2) immature fish respond abnormally to similar materials.

Goldfish *Carassius auratus*

Inasmuch as thousands of goldfish have been induced to spawn in various conditions at a number of hatcheries over a period of years the following method may be considered as standard. Almost identical techniques have been used with many carp and golden shiners.

During the regular goldfish spawning season from March to June, well-developed females 3 to 12 inches in total length usually spawn or can be hand-stripped within 12 to 20 hours after a single injection of pituitary taken from a fish of equal weight, or an injection of dried pituitaries of 1 to 5 milligrams per fish (2 to 3 milligrams per pound of body weight), depending on size. In practically all cases, fresh or acetone-dried materials in 1 cc. of distilled water were injected intraperitoneally at the axil of the pelvic fin of the female. Males were injected only to increase the flow of milt when hand-stripping was practiced.

Goldfish have responded to pituitaries from 14 species (table 4). Fish were spawned at temperatures from 52° to 85° F. For best results, water temperatures should be between 60° and 80° F. and an adequate supply of oxygen must be present. Goldfish were usually injected with the appropriate amount of pituitary materials in the afternoon, the dosage having been determined a day or two previously with the injection of some 10 or 15 females with varying amounts of the pituitary materials to be used. After the females were injected they were placed in concrete or metal troughs or in small earthen ponds with an equal number of males which may or may not be injected. Spanish-moss spawning mats were placed around the edges of the troughs and ponds, as described by Prather, Fielding, Johnson, and Swingle (1953). Such fish usually began spawning about daylight on the following morning and completed spawning by 10 or 11 a.m. Sudden drops in temperature from 70° to 50° F. usually did not completely inhibit spawning, but only delayed it. When spawning was finished, the mats were moved to a hatching pond which had been filled recently and was therefore free from predators. If the water had been standing for several days in the pond, or if runoff or pond water was used, this water was treated with 2.5 p.p.m. Lexone 2 or 3 days before stocking. This treatment effectively removes crayfish, backswimmers, some parasites, copepods, and predatory insect larvae. If properly used, Lexone will not be detrimental to the fertility of the eggs. The required number of eggs should be placed in the hatching pond in a single day, if possible, so that all fry will be the same age. Consequently the growth of fry will be more uniform, and some parasites, such as *Lernea*, will not be transferred from brood stock to fry. Any larvae of this parasite which might be transferred on the mats will likely die before the eggs hatch, since these larvae will live only about 24 hours in 2.5 p.p.m. Lexone and only a few days unattached to a proper host.[1]

An alternative method for the culture of this species involves the hand-stripping of eggs and sperm after injection. Instead of placing the female in spawning troughs or ponds, they are kept in holding tanks separated from males. Males are injected with pituitary materials to increase the amount of seminal plasma and provide more milt for the worker to use. The morning following the injections, the females are examined to determine their degree of ripeness. Those females that are ready to strip are separated into buckets or other convenient holding facilities. When several fish are ready to strip, the eggs are removed in a manner similar to that used for trout and pike. Each female is wiped dry with cheesecloth or toilet tissue, and the eggs are stripped into a plastic container, to which they do not stick as they do to glass or enamel pans. Great care should be taken to keep the eggs and milt perfectly free of water until completely mixed. The milt and eggs should be well mixed with a nylon bristle brush before any water is added. The procedure to this point should not be hurried, since the sperm of goldfish do not normally become motile until water is added.

We have kept goldfish eggs in a dry state outside the body of the female 1 hour (table 11) and found that they are totally receptive to fertilization. Also, goldfish eggs have been stored 1 hour in a sealed plastic bag in a refrigerator without serious loss of fertility (table 11). Shortly after mixing the eggs and sperm, small amounts of water are dripped from the finger into the eggs and sperm. After sufficient water is added, the eggs will begin to clump and adhere to each other. Slightly before much clumping begins, the eggs should be lifted with a nylon bristle paint brush and spread on spanish moss mats placed 4 to 6 inches under water in a holding trough or other convenient utensil. As the eggs are shaken from the brush, the water about the mat should be vigorously agitated to disperse the eggs over the mat. Also, the eggs can be poured from the plastic container and dispersed over the mat by vigorous agitation of the water. With a little practice the operator can achieve a dispersion of eggs similar to that attained by the fish under normal spawning conditions. Good dispersion of the eggs is essential; any dead eggs will become covered with fungus and smother adjoining living eggs if they are left in clumps or masses.

After the eggs have been placed on the mats, either of two incubation methods may be used. The mats of eggs may be placed in hatchery rearing ponds as described above, or they may be stored in regular hatchery receiving troughs which have flowing water. If many dead eggs are present, they may be flush-treated with malachite green at about 2 p.p.m. Water inflow should be adjusted so that the chemical is flushed out within an hour. When the eggs are eyed they may be moved from the troughs to a growing pond which has been properly prepared for fry. Moving the eggs at the eye stage is much easier than after hatching, since the fry of goldfish are small and delicate. We have never tried to feed or raise carp or goldfish in troughs. Trough-rearing may be feasible in this country in small hatcheries which rear fancy goldfish. The above methods have been used for several years to raise goldfish at commercial fish hatcheries with which we have been associated as employee or consultant. Also, these techniques have been used to raise young goldfish for food for bass at the National Fish Hatchery, Tishomingo, Okla., for the past four seasons. In one instance 675,000 goldfish weighing 2,925 pounds were reared in a 0.7-acre pond in 5 months. This concentrated population of fish did not suffer any serious diseases or mortality (Cozort, 1955).

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[1] John J. Giudice, Control of *Lernea carassii* Tidd, a parasite copepod infecting goldfish in hatchery ponds, with related observations on crayfish and the fish louse *Argulus* sp. Master's thesis, University of Missouri, 1950.

W. M. Tidd, Studies on the life history of a parasitic copepod, *Lernea carassii*. Doctoral thesis, Ohio State University, 1947.

Carp (*Cyprinus carpio*)

The technique for spawning carp is almost identical to that of goldfish (table 12) except that, in our experience, carp are refractory to heteroplastic injections. We have injected as many as 20 goldfish pituitaries in carp with no results. Similar doses of buffalo and catfish pituitaries also have been tried with negative results, whereas a single carp pituitary will induce female carp of the same group of fish to spawn. Ordinarily, carp are difficult to spawn in small earthen ponds or hatchery troughs; but under the influence of exogenous pituitary hormones, they can be effectively spawned in such ponds or troughs, or they can be hand-stripped as described above for goldfish.

Golden shiner (*Notemigonus crysoleucas*)

The technique described for small fish was used for this species and the three notropids following. The fish were anesthetized, held in the folds of a net, and the injection was made through the mesh. Female golden shiners received amounts of pituitary varying from one-twentieth to a whole pituitary from a 1-pound carp. One-tenth of a pituitary for gravid females 3 to 5 inches long is recommended. The eggs are adhesive and Spanish-moss mats were provided for spawning. If hand-stripping was employed the procedure outlined above for handling the adhesive eggs of goldfish was followed. At a water temperature of 68° F., spawning was induced in about 15 hours after a single injection. In addition to the above experiments, we have worked with commercial bait raisers, and the pituitary method was applied to large-scale operations. The brood fish were usually large, and anesthetics were not necessary. Most of this work was not subject to controls, but the method definitely appears suitable for the production of large numbers of golden shiners with fewer brood fish and less possibility of disease. Excellent production and uniformity in size of the bait minnows were achieved at Sulphur Fish Hatchery, Sulphur, Okla., when 200 brood females were injected and allowed to spawn in a natural situation in an earthen culture pond. Similar ponds stocked according to previously recommended methods produced inferior results.

Emerald shiner (*Notropis atherinoides*)

The emerald shiner was induced to spawn when injected with the water extract of one-tenth of an acetone-dried gland (estimated from 1 to 2 milligrams) from a buffalo (table 12). These shiners were collected from Lake Erie during the second week of July. Each of nine fish, 2 to 3 inches in length was anesthetized in 1:10,000 MS 222 for 1 minute and then was injected intraperitoneally with 0.1 milliliter of distilled water containing 100 units of crystalline penicillin-G plus the pituitary. The distended abdomens of three of the nine fish indicated that they were gravid females. One fish died the following morning; the others were alive and appeared normal. They were placed together in water at 76° F. in a 15-gallon aquarium. A piece of ⅛-inch mesh nylon net was hung between the fish and the bottom of the aquarium to prevent consumption of the eggs by the parent fish. The shiners spawned 20 to 24 hours after injection, and fertile eggs were found loose and scattered on the slate-bottomed aquarium. They were observed until developed to the primitive streak stage. No attempt was made to put the eggs in a proper hatching environment. Fungus attacked the eggs, and none hatched.

Mimic shiner (*Notropis volucellus*)

The dosages (table 12), injection technique, and aquarium setup was the same as for the emerald shiner, with the exception that the pituitary was from carp. Fish were collected from Lake Erie in the third week of June, and 18 fish about 2 inches long were injected. Six of these were known to be gravid females, but could not be hand-stripped before injection. No fish were lost within a 24-hour period after injection, but two died within the next 3 days. The eggs were loose and scattered, both individually and in clumps, over the bottom of the aquarium. They were observed to be fertile, but their development was not followed.

Redfin shiner (*Notropis umbratilis*)

Six male redfin shiners in breeding color were chosen and were not injected. Four gravid females were injected at the same dosage as the emerald shiner (table 12) about 9 p.m., July 17, and the males and females were placed together in a 5-gallon aquarium. The following morning between 7 and 10 o'clock spawning began and continued until about 4 p.m. It appeared that one male dominated the aquarium, driving the others to the surface to hide in the folds of the net while he spawned with the females. The dominant male was much more brilliant in color than the others, although they were all about the same color at the beginning of the experiment. (Later, in another experiment, a breeding male was placed with an injected female and within 2 hours the color on his fins had become much more intense.)

Eggs were deposited in a small pile in one end of the aquarium, and later in another pile at the other end of the aquarium. The selection of a new position at the far end of the aquarium away from the observer may have been caused by disturbances. Since the male was separated from the eggs by netting, it could not be determined whether or not he attempted to tend them. He moved throughout the aquarium. The eggs were left in the bottom of the aquarium. Although many of them became infected with fungus, several uninfected eggs hatched during the third day.

Channel catfish (*Ictalurus punctatus*)

The induction of spawning of the channel catfish by the injection of pituitaries has been previously described (Sneed and Clemens, 1960), and an abstract of this report is presented here for the sake of completeness.

Seventy-four pairs of channel catfish were injected with fish pituitary and induced to spawn in aquariums. Only the females were injected. Ten-gallon aquariums were the most satisfactory containers for spawning fish up to 2½ pounds in weight. Larger fish required proportionally larger containers. Peritoneal lesions and inflammation which often accompany intraperitoneal injections appeared to be controlled by including 10,000 units of crystalline penicillin-G with each injection.

Pituitaries from carp, buffalo, flathead and channel catfish, and gar were successfully used. There was little difference noted in the potency of the pituitaries from these different species, regardless of the month of collection. The total amount of acetone-dried pituitary material required to spawn channel catfish varied from 3 to 32 milligrams per pound of body weight of the recipient. Most females required 3 injections at 2 milligrams per pound of body weight. The total number of injections required varied from 1 to 28, with the average being 3. The period of time from the last injection to spawning varied from 2½ to 72 hours, but most fish began spawning 16 to 24 hours after the last injection.

Females believed to be refractory to spawning in the hatchery ponds and in their natural habitat were induced to spawn in aquariums by pituitary injections.

Flathead catfish (*Pylodictis olivaris*)

We have spawned four pairs of the flathead catfish, and the general technique was the same as for the channel catfish. The females were placed with males of similar size in 55-gallon aquariums, 24 x 25 x 14 inches. One pair was spawned in a cement trough. The fish were obtained from Lake Texoma on July 5 and injections began 2 days later. The data in table 13 outline the treatment used on individual fish to induce spawning and should serve as a starting point for those who wish to work with this species. Before flathead catfish can be spawned with any degree of success, some experience in selecting females is needed. Ripe females are usually darker in color than males, but were difficult to discern by external examination in the fish which we handled. The best criterion for evaluating the spawning condition of the females is the appearance of the genitalia. The genital papillae should be slightly raised, somewhat reddish, and the genital opening slightly dilated.

When examined in June, the ovaries of flathead catfish brood stock kept all winter in a hatchery pond were so poorly developed that we felt the fish could not be spawned. However, a hatchery in southern Texas that kept part of our brood stock through the winter was able to spawn one pair. Similar reports (personal communication) of difficulty in rearing flathead catfish brood stock have come to us from Arkansas and Alabama. Water temperatures and failure of the fish to feed properly in hatchery conditions on artificial foods may be major deterring factors in the sexual development of this species.

A local minnow breeder successfully spawned five pairs of flatheads with injections of pituitary materials according to our recommendations (Nos. 5–9 in table 13). He used buffalo, carp, and flathead pituitaries at dosages of 2.5 to 4 milligrams per pound of fish every 24 hours. Two of the females spawned with one injection, which suggests that flatheads respond to gonadotropins in a manner similar to other species if they are physiologically ready to spawn.

White bass (*Roccus chrysops*)

Two female white bass secured from the Red River below Lake Texoma on June 12 contained unspawned eggs, although the white bass normally finishes spawning before this time. These fish were taken to the hatchery with males of similar size. Both males and females were injected with ½ milligram of acetone-dried carp pituitary and placed in hatchery holding troughs, with Spanish-moss mats placed on the bottom and around the edges of

TABLE 13.—*Injection records of nine successful spawns of flathead catfish*

Fish	Number of injections	Donor species	Dosage (milligram per pound in 24 hours)	Female weight (pounds)	Remarks
No. 1	4	Flathead	4.0	9.0	Ovulation of a few eggs occurred without spawning; different male added before spawning was achieved.
No. 2	4	Buffalo	2.0	15.5	Spawned in cement tank.
No. 3	3	Carp	6.0	6.0	Partial spawn.
No. 4	4	Carp	2.0	6.8	Partial spawn.
No. 5	3	Buffalo and carp	------------	Est. 10.0	240 milligrams of B-12 with first injection; spawned in barrel in cement trough.
No. 6	5	Buffalo and flathead.	2.7	17.2	
No. 7	1	Buffalo	2.5	6.0	Collected in spawning condition, June 10, from Washita River; spawned in barrel in cement trough.
No. 8	1	Buffalo	2.5	1.8	Collected in spawning condition, June 10, from Washita River; spawned in barrel in cement trough.
No. 9	2	Buffalo	4.0	4.0	Collected in spawning condition, June 10, from Washita River; spawned in barrel in cement trough.

the trough (table 12). Seventeen hours later the fish were examined and were not ready to hand-strip, nor had any spawning occurred in the trough. The females were injected a second time with 2 milligrams of acetone-dried carp pituitaries and spawning occurred in the trough within 3 hours. Most of the spawn was placed on the mats, but there were many eggs on the sides and bottom of the troughs where the mats did not cover the entire surface. Had the bottom been completely covered with mats, 90 percent of the spawn could have been collected. Microscopic examination at the end of 12, 24, and 48 hours revealed about 60 percent of the eggs were fertile. These eggs were not observed further. One mat of eggs was placed in an earthen pond, but no fry were found, which may have been due to the fact that snails (*Physa*) soon ate the eggs.

White crappie (*Pomoxis annularis*)

Four pairs of white crappie were spawned in a cement trough containing Spanish-moss mats, and with a small flow of water running through the trough (table 12). One female received intraperitoneally one cholesterol pellet containing 1½ milligrams of an extract of chum salmon pituitary. At the end of 15 hours the genital pore was large and red; and at the end of 48 hours, a few eggs could be obtained by hand-stripping. This female was then injected with 4 milligrams of acetone-dried carp pituitary and spawned the following night when the water temperature was 61° F.

On another occasion, three pairs spawned within 30 hours after a single injection of acetone-dried carp pituitary. The eggs successfully hatched and the fry appeared normal.

Rock bass (*Ambloplites rupestris*)

A series of failures to induce spawning in nest building centrarchids has included the largemouth bass, bluegill, green sunfish, and rock bass. The information from these failures and from the successful spawning of two pairs of rock bass suggests a possible approach to a successful technique.

1. The condition of the male seems to be more important than in other species. It is the male that builds the nest. If the male does not initiate this behavior, then apparently spawning will not take place. For this reason, it may be good practice to inject the male, unless the male has been observed to exhibit some prespawning behavior.

2. The condition of the female is more difficult to assess because the genital openings reveal little concerning gonadal condition, and outward appearances of ovarian development can often be misleading.

3. Specialized environmental conditions

may have to be provided to give the necessary release to trigger the spawning. In the past, in spawning cyprinids, ictalurids, and serranids, the environmental conditions provided have been simple—space, oxygen, water exchange, and occasionally spawning mats. We would like to retain the idea (proposed in a previous paper, Sneed and Clemens, 1960) that any physical or chemical stimuli which normally cause the fish's own pituitary to secrete the gonadotropins necessary to precipitate the spawning act were bypassed, so to speak, by the exogenous gonadotropins. However, the successful spawning of two pairs of rock bass included some environmental additions which at this point seem necessary.

Two pairs of rock bass were spawned under similar conditions (table 12). A water temperature of 76° F., a coarse gravel bottom with a slight current of water flowing over the gravel, and a five-gallon aquarium constituted the facilities. Both males and females received two injections of buffalo pituitary 24 hours apart, amounting to a dosage of about 2 milligrams. Eggs were scattered over the gravel and were adhesive. They hatched in 3 days and were reared successfully.

Other experiments involved gravel and aeration (without running water), no gravel and running water, no gravel and aeration; each failed to induce spawning. However, two additional, unsuccessful attempts were made in what was considered to be favorable conditions. In these experiments, as well as in the other unsuccessful ones, the condition (gonadal development) of the fish may have been a limiting factor.

Zebra fish *(Brachydanio rerio)*

After a great deal of preliminary experimentation with zebra fish, a larger experiment was attempted. One hundred adult zebras were bought from a tropical fish supply house. Twenty-six females were selected, and 24 of these were injected with 0.6 milligrams of acetone-dried carp pituitaries in 0.2 milliliters of distilled water. The remaining 74 fish were either males or fish difficult to sex. Ten injected and two control females were placed in individual spawning containers (battery jars), with plastic screen suspended two inches from the bottom to protect the eggs. Fourteen injected females were placed together in an aquarium. Two fish believed to be males were stocked per female; i.e., 28 such fish were used. None of the males were injected. Injections were made at 6 p.m., and four females died shortly after being injected. The following morning, four of the six living females in individual containers had spawned a total of 340 eggs (table 14), while one of the uninjected females had spawned. The spawning of one of the control females considerably weakens, in this instance, the case for inducing zebra fish to spawn. However, since this species readily spawns in small containers, the difference in injected fish over the controls will never be as clear cut as in fish with more precise spawning requirements, such as channel catfish, rock bass, etc. The egg count of the females that spawned individually, including the two which did

TABLE 14.—*Results of injecting zebra fish with 0.6 milligram of acetone-dried carp pituitary in 0.2 milliliter of distilled water*

Number of females	Number of males per female	Number of ovulating females	Number of fertile eggs	Total number of eggs	Number of females dead	Percent of fertile eggs
Selected brood stock:						
1	2	1	93	112	-------	83.1
1	2	1	0	1	-------	0
1	2	0	0	2	[1] 5	0
1	2	0	33	65	-------	50.7
1	2	1	121	143	-------	84.6
1	1	0	11	17	-------	64.7
1 (control)	2	0	0	0	-------	0
1 (control)	2	0	50	52	-------	96.1
14	1	4	272	366	-------	74.3
Culls: [2]						
33	-------	-------	116	121	14	95.8

[1] Four of these fish died shortly after injection and they were not included in the remainder of the data.

[2] These fish were not sexed.

not spawn, averaged 57 eggs per female with a range from 17 to 143 eggs. On the basis of this average, about 6 of the 14 females spawned in the aquarium experiment.

The culls from this lot of fish were injected with the same amount of pituitaries of the same pituitary lot. In this experiment both males and females were injected because the poorly developed females and the more immature fish were difficult to sex. Eleven of 33 fish died immediately after injection, 3 died the following day, and several others were rather sluggish. The fish first lost their equilibrium and their ability to swim; their scales began to protrude; and in the final stages, the body became rigid. From the remaining 22 injected fish, 121 eggs were obtained, a yield of 11 eggs per female if a 1:1 sex ratio existed.

The percent of fertile eggs found in each spawning container varied from 51 to 96, the highest being in the uninjected control fish and in the culls. There are a number of possible reasons for the lowered fertility: (1) the exogenous gonadotropin might have had a deleterious effect on the eggs; (2) the amount of gonadotropin injected might have been excessively high and caused the female to void her eggs too rapidly and they settled through the screen before the male could fertilize them; (3) the males were in poor condition and incapable of fertilizing many eggs; (4) the number of males required to spawn a female and fertilize the eggs was inadequate.

The fact that the percentage of fertility in the eggs from the cull fish approximates that in the controls suggests that exogenous gonadotropins do not necessarily lower the fertility and that the lower fertility in the eggs of the select fish was perhaps due to an inadequate number of males. It may be wise to stock males and females in ratios greater than 1:1 or 2:1, and place the plastic netting near the bottom to insure proximity of the eggs and sperm.

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EQUILIBRIUM YIELD AND MANAGEMENT OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

March 3, 1849

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 62

RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two Bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

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EQUILIBRIUM YIELD AND MANAGEMENT OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

Norman G. Benson and
Ross V. Bulkley
Fishery Research Biologists

RESEARCH REPORT 62

UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, *Secretary*
Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*
FISH AND WILDLIFE SERVICE
Clarence F. Pautzke, *Commissioner*
BUREAU OF SPORT FISHERIES AND WILDLIFE
Daniel H. Janzen, *Director*

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ABSTRACT

Equilibrium yield of the cutthroat trout, *Salmo clarki lewisi* Girard, in Yellowstone Lake, Wyo., is determined from data on catch and spawning runs from 1945 to 1961. Changes in growth rate, spawning runs, mortality rates, and year-class strength are related to differences in total catch. Three stages of exploitation of the stock are defined, and the maximum safe catch or equilibrium yield is estimated at 325,000 trout. Management of the sport fishery according to equilibrium yield is discussed with reference to regulations, distribution of fishing pressure, planting, and interspecific competition. The Yellowstone River fishery is treated briefly.

EQUILIBRIUM YIELD AND MANAGEMENT OF CUTTHROAT TROUT IN YELLOWSTONE LAKE

By Norman G. Benson and Ross V. Bulkley, *Fishery Research Biologists*
Rocky Mountain Sport Fishery Investigations, Logan, Utah

Equilibrium yield is one of the primary objectives of trout fishery management where wild fish are involved. This report presents a method for estimating maximum equilibrium yield and discusses the relations of yield to management of the stock of cutthroat trout (*Salmo clarki lewisi* Girard) in Yellowstone Lake, Wyo. The study is based mainly on data collected from 1945 to 1961 on the catch and spawning runs, but it includes other biological information on this trout population. Natural fluctuations in stock abundance are distinguished from those caused by fishing, and past management is evaluated.

Yellowstone Lake, in Yellowstone National Park, has one of the few native cutthroat trout stocks in the United States that have not had to compete with large populations of introduced fishes. The lake and tributaries make up a closed water system, as Yellowstone Falls (180 feet high) is located 15 miles below the lake. The National Park Service has prevented extensive development of the lake shore, and the lake ecology has remained relatively unaltered.

This study was essentially confined to the management of Yellowstone Lake, but the fishery in the 15 miles of the Yellowstone River from the outlet to the falls has also been reviewed.

The scope of the material in this report makes it advisable to describe briefly the subjects considered and their order of presentation. First, general background material is presented on the history of fishery investigations on Yellowstone Lake, fish cultural work, description of Yellowstone Lake, life history of cutthroat trout, and lake stocks. Second, the catch from 1950 to 1961 is discussed; this includes the number of fish caught, catch per man-hour, lengths, ages, and sexual maturity. Third, we discuss the changes in growth rate, spawning runs, mortality rates, and year-class strength during the study period and consider the causes thereof; changes caused by fishing are differentiated from those produced by natural conditions. Fourth, methods and calculation of maximum equilibrium yield are presented. Fifth, the management of this fish population according to equilibrium yield is discussed in respect to regulations, distribution of fishing pressure, interspecific competition, and planting. The last section includes the biology and management of the Yellowstone River fishery. Much of the data and some methods used are put in the appendixes to avoid complicating the presentation.

The manuscript was reviewed by Drs. Oliver Cope, William Sigler, John Neuhold, William Ricker, and Kenneth Watts, and by Orville Ball. Their suggestions and criticisms are gratefully acknowledged.

The authors are now at the Bureau's North Central Reservoir Investigations, P.O. Box 139, Yankton, S. Dak.

YELLOWSTONE LAKE FISHERY

BACKGROUND DATA

Investigations

The cutthroat trout population in Yellowstone Lake was first mentioned by Hayden (1872), who reported that a single species of fish was in the lake, a trout which weighed from 2 to 4 pounds each. Jordan (1891) made a reconnaissance survey of the lake in 1889, and Evermann (1893) described the Yellowstone cutthroat trout in detail. Kendall (1915) and Smith and Kendall (1921) made further general studies on the biology of the cutthroat and its management. Unpublished reports were prepared by Schultz (1936),[1] Madsen (1940),[2] and Foster (1941).[3]

The U.S. Fish and Wildlife Service conducted more detailed biological studies from 1943 to 1946 on spawning runs and stocking methods under the direction of Dr. Stillman Wright, but the first detailed investigation on this cutthroat trout population was carried out by the Rocky Mountain Investigations of the Service from 1949 to 1961. Some recent publications dealing with the biology of the cutthroat and lake ecology are by Laakso and Cope (1956), Cope (1953, 1956, 1957a, 1957b), Ball (1955), Ball and Cope (1961), Benson (1960, 1961), Bulkley (1961), and Bulkley and Benson (1962).

Fish culture

A U.S. fish cultural station was operated on Yellowstone Lake from 1901 to 1953. Fish were trapped in tributary streams during their spawning runs, and eggs were collected and fertilized. Most eggs were used for stocking other waters in and outside Yellowstone National Park, but eyed eggs, advanced fry, and fingerlings were also planted in Yellowstone Lake and tributaries. The fingerlings were fed only a short time before stocking and were in the 1-inch class. A summary of the egg take and stocking in Yellowstone Lake and tributaries from 1940 to 1953 is shown in table 1. The number of spawners allowed to spawn naturally varied greatly from year to year and from stream to stream. Influences of planting and egg collecting on the fish stock are discussed later. Several programs to evaluate these plantings by fin clipping were never completed.

Table 1.—*Number of cutthroat trout eggs collected from tributaries of Yellowstone Lake and number planted in lake or in tributaries, by years, 1940 to 1953*

[In thousands]

Year	Number of eggs collected	Number planted in lake or tributaries		
		Eyed eggs	Advanced fry	Fingerlings
1940	43,000	963	3,963	1,752
1941	39,000	1,251	4,175	1,661
1942	25,000	2,001	3,339	1,567
1943	25,000	2,061	3,161	1,615
1944	24,000	800	3,200	1,125
1945	20,000	0	2,600	602
1946	24,000	0	3,075	608
1947	21,000	0	2,980	403
1948	19,000	0	550	0
1949	19,000	0	742	0
1950	18,000	625	1,011	0
1951	18,000	0	0	0
1952	15,000	0	0	0
1953	8,000	0	0	0

Native and introduced fishes

Except for the cutthroat trout, the only endemic species in the drainage is the longnose dace (*Rhinichthys cataractae* Valenciennes). The mountain whitefish (*Prosopium williamsoni* Girard) was stocked in the Yellowstone River above the falls in 1898 but did not survive. In 1908 and 1909, landlocked salmon (*Salmo salar sebago* Girard) and rainbow trout (*Salmo gairdneri* Richardson) were introduced into Yellowstone Lake, but neither had survived to 1915 (Kendall, 1915). Exotic fishes presently in the lake are the longnose sucker (*Catostomus c. catostomus* Forster), introduced about 1923, the redside shiner (*Richardsonius balteatus hydrophlox* Cope), introduced before 1955, and the lake chub (*Hybopsis plumbea* Agassiz), introduced before 1949. The longnose sucker is the only nontrout species widely distributed in the lake, but it is restricted to the littoral zone. The other species are confined to shallow lagoons, tributary streams, or protected shallow areas. The relations of the sucker and redside shiner populations to the cutthroat trout stock are discussed later.

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[1] "Report of observations on the cutthroat trout of Yellowstone Lake in regard to stocking," by Leonard P. Schultz. U.S. Bureau of Fisheries typewritten report, August 18, 1936, 12 pp.

[2] "Report on age and growth of the cutthroat trout (*Salmo lewisi*) of Yellowstone Lake, Wyoming," by M. J. Madsen. U.S. Bureau of Fisheries typewritten report, 1939.

[3] "Preliminary report of Yellowstone Lake investigations, season of 1940," by Richard F. Foster. U.S. Fish and Wildlife Service typewritten report, plus 5 progress reports, 1941.

Regulations

Fishing regulations before and during the 1920's allowed a limit of 20 fish per angler per day and limited fishing methods to hook and line. Following an increase in fishing pressure in the 1930's and until 1949 the daily limit was 10 fish. This limit was lowered to 5 fish in 1949 and to 3 fish in 1953. Length limits have never been used. Before 1953 the fishing season extended from May 30 to October 15. Since then the opening day has been June 15 on the lake and June 1 on the Yellowstone River; the closing day is October 15.

Yellowstone Lake

Yellowstone Lake (fig. 1) has a surface area of 136.7 square miles, a mean depth of 139 feet, a maximum depth of 320 feet, and a shoreline development of 3.04 (Welch, 1948). It lies at an elevation of 7,731 feet. The lake basin is volcanic in origin, and the limnological characteristics can best be described as oligotrophic. Total alkalinity ranges from 30 to 38 p.p.m., and conductivities (reciprocal megohms at 25° C.) range from 62 to 123. The lake freezes over each year from January to May, and summer surface temperatures rarely exceed 65° F.

Principal bottom organisms are *Gammarus lacustris* (Sars), *Hyallela azteca* (Saussure), and several species of tendipedids. The phytoplankton is dominated by diatoms, but a pulse of *Anabaena* occurs each August. The most abundant zooplankters are *Diaptomus shoshone* (S. A. Forbes), *Daphnia schoedleri* (Sars), and *Conochilus unicornis*. Plankton and bottom fauna species composition has not changed, to any measurable degree, from the time of an early reconnaissance study by Forbes (1893) in 1890 to the present time. Periodic temperature records from the Yellowstone Fish Cultural Station from 1920 to 1953 have also emphasized the stability of this environment. We have considered the lake environment as a constant when annual differences in the statistics of the trout stock are analyzed.

Fourteen major and 26 minor tributaries empty into the lake. Yellowstone River is the largest stream entering the lake and is the stream that drains the lake. Most tributaries are cold, clear alpine streams, although hot springs drain into several tributaries.

Life history of cutthroat trout

The life history of the cutthroat trout in Yellowstone Lake has been described in several of the papers cited previously and is reviewed briefly here. Cutthroat trout of age groups III to VII, but predominantly fish of age groups IV and V, migrate into tributary streams of Yellowstone Lake and the Yellowstone River from May through July to spawn. Studies with tagged trout from 1951 to 1953 suggest that up to 98.78 percent of the spawning fish are initial spawners (Ball and Cope, 1961). Repeat spawning in alternate years occurs over four times more frequently than in consecutive years. An average of 48 percent of the spawners die in the spawning streams from predation and other causes; the survivors move back into Yellowstone Lake within a few weeks after they enter the stream.

Eggs are deposited in gravel from a few days to several weeks after spawners enter the streams, and fry emerge from the gravel an average of 30 days after egg deposition. Some immatures migrate to the lake within 2 weeks after emergence, while

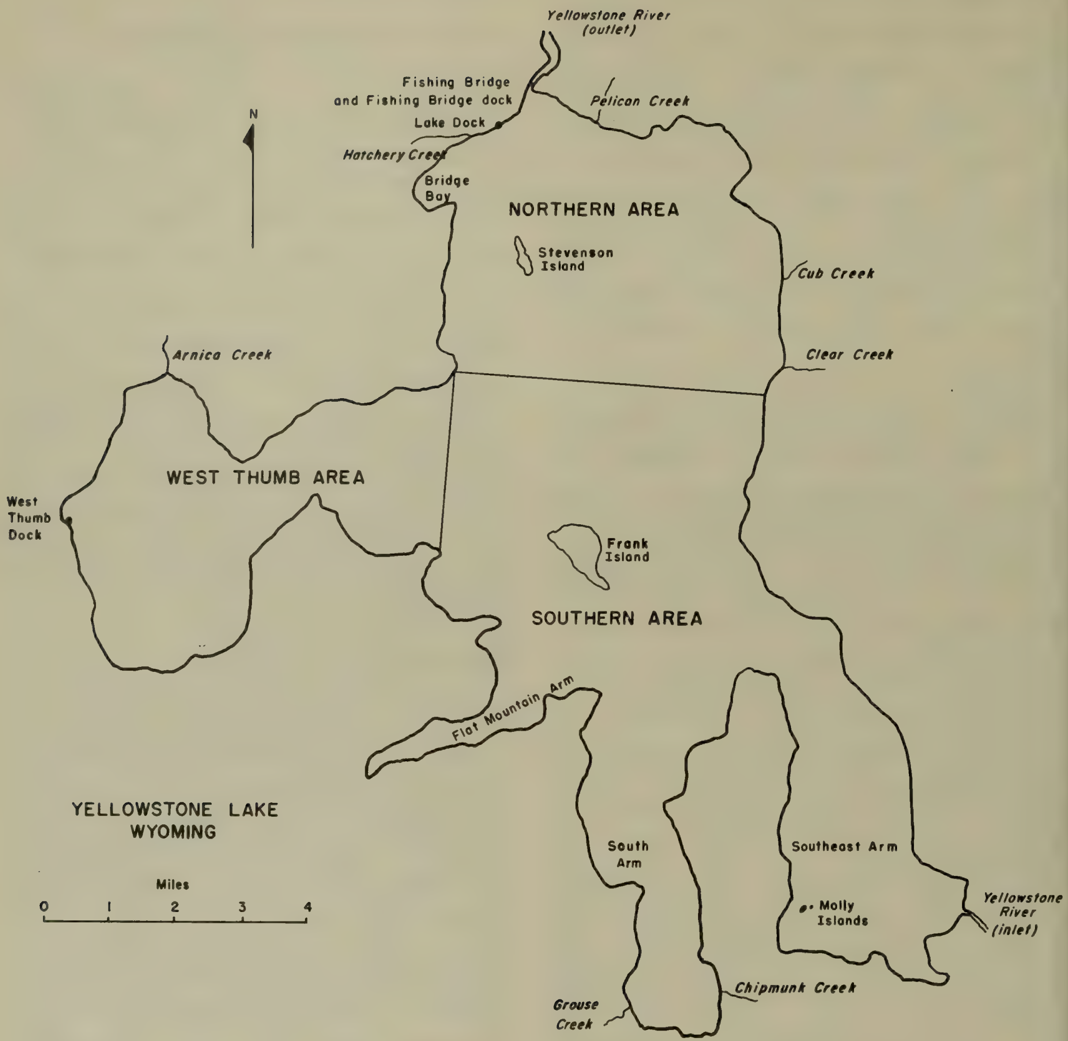

FIGURE 1.—Yellowstone Lake, showing areas and streams included in the study.

others remain in the streams for periods up to 2 years. Predation and other factors cause an estimated average mortality of 99.6 percent from the egg potential of the spawning population to the number of immatures that enter the lake. Mortality studies in Arnica Creek suggested a relatively high survival rate of 36.8 percent from the time that immatures leave the parent stream until they return as spawners. Both immature and adult trout showed strong homing tendencies (Ball, 1955).

Trout feed almost entirely on plankton and bottom fauna in the lake. Only 6 out of 694 stomachs examined contained fish (small cutthroat trout). There is a gradual transition from a plankton-bottom fauna diet to a diet predominantly of benthos in fish within the length range of 225 to 300 mm. Most trout are in the upper 45 feet of water during the summer, and few venture below the 75-foot depth. Small trout inhabit both the limnetic and littoral zones, while large trout are found predominantly in the littoral zone.

Fishing in spawning streams, except for the lower Yellowstone River, is not common, and the catch is taken almost entirely from the lake. A few trout enter the catch at age group II, but most trout caught are in age groups III, IV, and V.

Predation

The rate of natural predation in Yellowstone Lake must be considered when evaluating the causes of fluctuations in trout abundance over a period of time. The white pelican is the principal predator, but the black bear is also involved. Black bears consume a large number of trout during spawning, but the number of bears around Yellowstone Lake is maintained at a relatively constant level by the National Park Service. Double-crested cormorants are not common enought to be considered. Ball and Cope (1961) studied pelican predation and showed how it was related to spawning runs of trout. They suggested that the mortalities caused by pelicans alone exceeded fishing mortalities from 1950 to 1953.

Pelican predation will vary from year to year according to the number of pelicans. Pelican breeding is confined to the Molly Islands on Yellowstone Lake, and the number of young produced on these islands has been recorded by the National Park Service for some years. We have used these counts as a measure of the variation in amount of pelican predation. Murphy[4] reviewed the pelican count data from 1929 to 1959 (table 2), but stated that the accuracy of some of the counts may be open to question. He found that the number of young hatched increased during the 1930's to a high in 1946. Except for a low of 58 in 1950 the number hatched has ranged from 200 to 300 since 1946. He attributed the low numbers in 1931 and 1950 to normal population fluctuations, but the overall increase may be due to more rigorous protection on a national level. The data since 1950 do not suggest any significant changes in the pelican population, and we have not considered natural predation by pelicans as a variable in our analyses.

TABLE 2.—*Hatch of white pelicans on Molly Islands, for specified years, 1929 to 1960*

Year	Count of recently hatched birds
1929	134
1930	170
1931	54
1932	100
1936	180–200
1946	374
1947	228
1949	255
1950	58
1951	200 (banded)
1952	300 (est.)
1959	257
1960	[1] 275

[1] Data courtesy of Robert McIntyre, Chief Park Naturalist, Yellowstone National Park.

Fishing stocks

The term "stock" in this paper refers to a group of subpopulations as defined by Marr (1957). His definition of a subpopulation could apply to the progeny of each spawning stream in Yellowstone Lake, since the homing instinct is strong.

Analyses of the lake population would be simplified if all data collected could be viewed as coming from a single stock, but several factors have made it advisable to consider the northern area and West Thumb as two distinct fishing stocks (fig. 1). These two stocks were studied separately because (1) each is supplied largely from a different group of spawning streams as shown from tagging of post-spawners, (2) they differ in strength of year classes, (3) they differ in seasonal recruitment into the fishery, and (4) they differ in age composition of the catch. There is not enough fishing pressure in the southern area at the present time to define it as a fishing stock. Some of the differences between stocks are described below, but more complete data are presented later.

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[4] "The Molly Island nesting colonies of Yellowstone Lake," by Joseph R. Murphy. Unpublished mimeographed report to National Park Service, 9 pp. 1959.

The relations between the trout from five spawning streams and these two fishing areas from 1949 to 1953 were determined from tagging. Ball and Cope (1961) studied the dispersal of 18,836 tagged postspawners from five spawning streams (fig. 1). Trout captured during the same year as they were tagged were found closer to the spawning stream than those captured during the second or third year, and results from all years showed the same general movements. Trout tagged in Pelican Creek, Clear Creek, lower Yellowstone River, and near Fishing Bridge were primarily captured in the northern area. Trout tagged in Chipmunk and Grouse Creeks were often captured in the lightly fished southern area, but a large number were recovered in West Thumb during August of the year of tagging or the following year. Most Arnica Creek tags were recovered in West Thumb. Assuming that the movement of postspawners is similar to that of prespawners, the northern area stock is composed mainly of fish spawned near the location of fishing, while the West Thumb stock consists of locally spawned fish plus fish from spawning streams in the southern part of the lake.

Year-class strength of the catch varies widely because of this difference in the source of fish contributing to the catch in the two areas. Bulkley and Benson (1962) analyzed the causes and variance of the strength of year classes in Pelican and Chipmunk Creeks. The strength of year classes of spawners at Pelican Creek is correlated significantly ($r=.825$, $d.f.=6$) with the catch at Fishing Bridge, and heavy exploitation in the northern area influenced the Pelican Creek spawning runs. Pelican Creek is not as important a spawning stream as the Yellowstone River for the northern area, but factors influencing year-class strength in Pelican Creek must be similar to those influencing the entire stock of the northern area. The West Thumb catch is derived from a large number of relatively small spawning streams, and the year-class strength of the catch does not follow closely any of the sampled streams. The reason for the close agreement in year-class strength between spawning streams and catch in the northern area and the lack of a similar agreement in West Thumb is suggested in data from Bulkley (1961). Spawning streams that are in close proximity to each other are more similar in the strength of their year classes than streams that are widely separated.

The West Thumb catch is rather evenly distributed throughout the fishing season, while the catch in the northern area is heaviest during the spawning period and decreases rapidly after the middle of July. The northern area catch has more old fish than West Thumb because it is more dependent on spawning migrations.

CATCH

Number caught

Early records of the sport fish catch from Yellowstone Lake cannot be considered too reliable, since they were collected by interviews at the gates of the park. They give a general picture of the fishing intensity and show the increase from 1925 to 1944. The average annual catches for 5-year periods were as follows: 1925–29, 69,000; 1930–34, 93,518; 1935–39, 140,697; and 1940–44, 117,697.

An intensive creel census (Moore et al., 1952; Cope, 1957a) was conducted from 1950 to 1961. This census sampled segments of the fishery such as shoreline, rowboats, trailer boats, etc., separately, and the total catch and fishing effort is estimated from the sum of all segments. From 1950 to 1959 the catch increased from 200,015 to 393,497 fish and the fishing pressure from 292,989 to 526,728 hours (table 3). The increase was continuous except for 1953 and 1954, when the catch decreased owing to the lowering of the creel limit from 5 to 3 fish. Both the catch and the fishing pressure dropped in 1960. The drop in 1960 was related to a general decrease in fishing pressure and was not due to poor fishing. Fishing pressure in hours fluctuated less regularly than catch, but varied from 292,989 in 1950 to 526,728 in 1959.

TABLE 3.—*Estimated catch by areas and for entire lake and estimated fishing pressure for entire lake, by years, 1950 to 1961*

Year	Number of trout caught				Total hours of fishing
	Northern area	West Thumb area	Southern area	Entire lake	
1950	144, 816	48, 575	6, 624	200, 015	292, 989
1951	147, 999	52, 085	8, 171	208, 255	353, 090
1952	170, 255	63, 885	11, 137	245, 277	402, 989
1953	129, 529	38, 819	27, 525	195, 873	332, 684
1954	148, 171	53, 702	14, 060	215, 933	333, 004
1955	191, 647	78, 276	16, 133	286, 056	445, 206
1956	182, 999	86, 472	20, 750	290, 221	500, 799
1957	187, 462	92, 174	21, 519	301, 155	418, 963
1958	219, 036	105, 155	24, 836	349, 027	474, 951
1959	250, 310	117, 171	25, 986	393, 467	526, 728
1960	181, 588	85, 953	27, 357	294, 898	407, 327
1961	192, 211	97, 114	23, 269	312, 594	497, 664

Fishing pressure on Yellowstone Lake is not distributed evenly, owing to lack of easy access to the southern parts of the lake. Since the creel-census information was collected by sampling segments of the fishery separately, it was possible to break down quite accurately the catch rom the three areas of the lake designated n figure 1 (method in appendix A). From 1950 to 1960 an average of 65.5 percent of the cutthroat trout were caught in the northern area (34.7 square miles), 27.6 percent in West Thumb (26.1 square miles), and 6.9 percent in the southern area (75.8 square miles). Although the catch has increased more in West Thumb and in the southern area than in the northern area in recent years, the overall distributional pattern has not changed greatly.

Catch per man-hour

Royce and Schuck (1954), Schaefer (1957), and other workers have used catch per unit of effort as an important statistic for calculation of stock density and for management purposes. We have found catch per man-hour to be an unreliable index of stock density in the Yellowstone Lake fishery because there were too many factors other than stock density that influenced rate of catch. Catch per man-hour did not vary greatly from 1950 to 1961 even with a tremendous increase in catch (table 4). For shoreline anglers, it was usually about 0.5 fish per hour, and for boat fishermen it varied from 0.5 to 1.0 fish per hour. There were no consistent relations between catch per man-hour and total catch for rental and guide boat anglers, shoreline anglers, and private trailer boat anglers. This lack of relation could not have been due to error in the collection of data for the shoreline fishery because census clerks actually observed fishermen and did not

TABLE 4.—*Catch and catch per man-hour by rental and guide boats, shoreline fishermen, and private trailer boats, by years, 1950 to 1961*

Year	Rental and guide boats		Shoreline		Private trailer boats	
	Number of trout caught	Catch per man-hour	Number of trout caught	Catch per man-hour	Number of trout caught	Catch per man-hour
950	83, 351	0. 700	55, 370	0. 649	50, 377	1. 020
951	90, 442	. 670	48, 966	. 406	54, 857	. 980
952	102, 210	. 722	53, 423	. 404	75, 457	. 802
953	62, 364	. 659	64, 116	. 522	50, 118	. 680
954	72, 369	. 737	71, 919	. 616	51, 222	. 680
955	89, 470	. 756	97, 831	. 559	80, 270	. 698
956	94, 315	. 898	65, 383	. 342	110, 937	. 658
957	104, 786	. 816	63, 286	. 536	115, 141	. 808
958	93, 354	. 752	81, 210	. 530	149, 667	. 765
959	105, 277	. 895	140, 102	. 603	124, 421	. 718
960	76, 120	. 924	65, 539	. 503	126, 109	. 787
961	99, 795	. 856	98, 954	. 560	93 632	. 541

depend upon interviews. Two factors that were known to have seriously influenced catch per man-hour in the Yellowstone fishery were year-to-year variations in availability of the stock and a major change in fishing gear during the study period.

Most of the angling pressure on Yellowstone Lake is exerted on fish highly concentrated near shore in spawning migrations. Each year almost half of the catch from the shoreline and from rental boats at Fishing Bridge has been made during the first 4 weeks of the angling season (table 5). As the fish disperse after spawning they become more difficult to locate, and catch per man-hour decreases. Timing of the spawning period in relation to opening of the fishing season seriously influences the seasonal catch per man-hour, although this timing factor cannot be accurately evaluated. In years such as 1954, when spawning was delayed, opening of the fishing season coincided with the peak of the spawning period, and catch per man-hour remained high until the spawners dispersed. In years when environmental conditions were suitable for early spawning, such as 1951, many fish had spawned and commenced dispersing or had passed into spawning streams closed to angling before the fishing season opened. Catch per man-hour was correspondingly lower in these years, not because of reduced stock density in the lake, but because of earlier dispersal from the fishing area of a highly concentrated segment of the population.

Unusually high water levels in 1956 influenced catch per man-hour of shoreline fishermen, although the influence of water levels was not clearly evident during other years (fig. 2). Water levels in 1956 probably reduced availability of the cutthroat trout to shoreline anglers only, as boat fishermen did not experience a drop in rate of catch that year (table 4).

A major change in angling gear also occurred during the study period, with an ensuing increase in gear efficiency. From reports of concessionaires and sporting goods dealers, spinning gear was first introduced into the Rocky Mountain area around 1949. It did not become common on Yellowstone Lake until 1953 and 1954. The increase in catch per man-hour by shoreline fishermen after 1953 shown in table 4 is believed to have been caused partly by the increase in use of spinning gear. Spinning gear allows an angler to cast farther from shore with lighter lures. The introduction of newly designed fishing lures may also have influenced catch per man-hour, but the amount of influence is unknown.

Bulkley (1961) showed that growth rates increased from 1950 to 1956, and he could only attribute this increased growth rate to decreased stock density. Additional data presented in this paper support

TABLE 5.—*Percentage distribution by 2-week periods for anglers, catch, and catch per man-hour of shoreline and Fishing Bridge rental boats, 1954, 1955, and 1956*

[Angler and catch data given as percentages]

Two-week period	1954			1955			1956		
	Anglers	Catch	Catch per man-hour	Anglers	Catch	Catch per man-hour	Anglers	Catch	Catch per man-hour
June 15–28	18. 1	19. 5	0. 97	20. 0	21. 9	0. 63	16. 3	23. 1	0. 62
June 29–July 12	26. 0	28. 0	. 79	25. 3	25. 7	. 69	21. 9	23. 2	. 62
July 13–26	18. 5	20. 0	. 44	22. 8	24. 0	. 51	17. 4	21. 2	. 40
July 27–Aug. 9	20. 4	16. 3	. 39	12. 7	12. 3	. 37	26. 8	15. 3	. 28
Aug. 10–23	7. 9	10. 2	. 32	12. 5	10. 2	. 26	12. 5	13. 4	. 27
Aug. 24–Sept. 6	9. 1	6. 0	. 24	6. 7	5. 9	. 24	5. 1	3. 8	. 18
Total number	87, 433	137, 321	----------	131, 358	171, 988	----------	160, 457	147, 761	----------

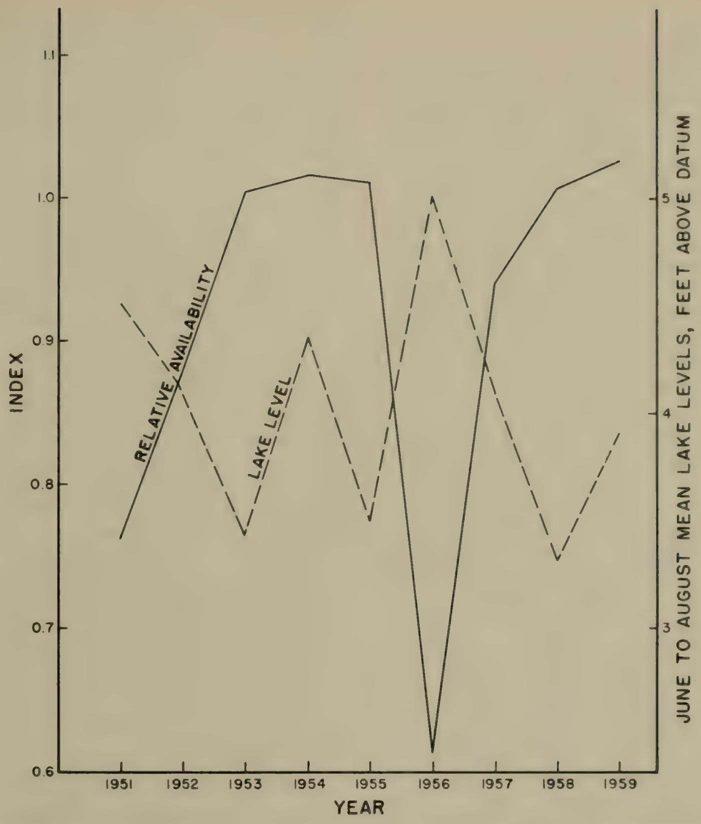

FIGURE 2.—Availability of cutthroat trout to shoreline anglers on Yellowstone Lake (solid line) as compared with mean monthly lake water levels from June to August (broken line), 1951 to 1959. Lake levels in feet above datum. Relative availability index from Rounsefell and Everhart (1954, p. 99).

his conclusion. Catch per man-hour has not decreased with a lower stock density, because as stock density has decreased gear efficiency has increased. Variations in timing of the spawning period and in water levels have further clouded the relation between catch per man-hour and stock density. These factors have made it impossible to utilize catch per unit of effort as a reliable index to the condition of the stock in Yellowstone Lake.

Lengths

Cope (1953) discussed mean total length measurements of the catch from 1950 to 1952 and found significant variations among years. Lengths from 1950 to 1961 show similar variation (table 6), and the causes of these variations are shown later to be related to growth rate, age composition, and year-class strength. The significant point on length data is that most of the catch during all years was between 300 and 400 mm. in total length. The means varied from 336.7 to 357.8 mm., and the means plus or minus one standard deviation ranged from 296.7 to 396.8 mm. Few fish were caught below 200 mm. or above 500 mm. Thus the stock available for sport fishing exploitation is restricted to a relatively small length range. The maximum length is limited by the maximum size attainable in the stock, and the minimum length is limited by the low availability of fish below 275 mm.

Age composition

The scale samples from Fishing Bridge dock have been assumed to represent the entire northern area stock. Several scale-sample collections from fishing areas other than Fishing Bridge in the northern area (*e.g.* Lake Dock, Bridge Bay) were compared with the Fishing Bridge samples, and no significant differences were found. The West Thumb dock samples have been applied to the entire West Thumb stock for the same reason.

Table 6.—*Total length of cutthroat trout caught from Yellowstone Lake, by years, 1950 to 1961*

[In millimeters]

Year	Number of trout in sample	Mean total length	Range in length		Standard deviation
			Minimum	Maximum	
1950	1,931	345.1	205	470	28.2
1951	1,063	343.2	215	455	39.3
1952	1,061	336.5	205	500	39.8
1953	711	345.7	195	470	39.5
1954	1,395	337.7	205	450	38.1
1955	1,946	343.6	200	505	38.7
1956	1,668	348.7	210	455	34.3
1957	634	353.3	205	455	33.5
1958	676	357.8	185	480	39.0
1959	2,349	353.9	215	470	38.4
1960	1,683	346.3	210	450	38.5
1961	2,478	344.2	225	460	36.2

Bulkley (1961) analyzed the age composition of the catch from 1950 to 1959, and his data are summarized as follows (table 7): (1) Age groups IV and V make up most of the catch, although age group III was significant in all years; (2) there were more older fish caught at Fishing Bridge than at West Thumb because a large part of the former fishery is dependent upon a spawning concentration; (3) relative abundance of age groups in the catch from both areas up until 1957 was related to year-class strength; and (4) there were fewer fish of age groups VI and VII in the catch after 1953 than in earlier years.

The difference in age composition between the West Thumb and Fishing Bridge fisheries is of a seasonal nature since spawning concentrations are largely dispersed after the middle of July (table 8). Up until July 11, fish of age groups V, VI, and VII are much more common at Fishing Bridge than at West Thumb, but there are no consistent differences between areas after that time. Fish of age groups II and III increase in importance as the season progresses, owing to recruitment.

Age compositions of the northern area and West Thumb catches from 1950 to 1961 are presented in figure 3. Before 1956, age composition of the northern area catch was not related to size of catch. The 1948 year class was strong at age IV and weak at age V. The 1949 year class contributed strongly at age III, weakly at age IV, and strongly at age V. The 1950 and 1951 year classes contributed weakly at ages III and IV, but strongly at age V. This haphazard entrance of age groups into the catch is probably indicative of a lightly exploited fishery. It also indicates varying rates of exploitation during these years. Stroud's (1948) data on Norris Reservoir in Tennessee showed a similar lack of trends, and this reservoir was known to be lightly exploited. Ricker (1954, p. 583) described similar oscillations for multiple-age spawning stocks.

Beginning in 1956, the catch of age group IV fish began to increase significantly, and this trend continued until 1959. The 1953 year class entered the catch strongly at ages IV and V. The 1954 and 1955 year classes entered the catch strongly at age IV but weakly at age V. The 1956 year class entered the catch strongly at age III, and the 1957 year class entered the catch strongly at ages II, III, and IV. The 1958 year class also entered the catch strongly at age III.

In the West Thumb fishery, the catch of age group IV fish increased steadily from 1953 to 1959, which coincided somewhat with the increased catch during those years. The 1958 West Thumb catch contained the largest recorded number of age group V fish, followed by 3 years with relatively few age group V fish in the

TABLE 7.—*Age composition of catches from Fishing Bridge and from West Thumb docks, by years, 1950 to 1961*

Year of capture and location	Percent in age group—						Mean age (years)	Mean length (mm.)
	II	III	IV	V	VI	VII		
950:								
Fishing Bridge	1.0	13.1	37.9	37.2	10.1	0.7	4.44	358.9
West Thumb	6.4	34.2	24.8	30.4	4.0	.2	3.92	338.2
951:								
Fishing Bridge	2.0	13.5	34.0	36.3	13.5	.7	4.48	349.6
West Thumb	1.6	33.9	40.7	21.3	2.2	.3	3.90	337.8
952:								
Fishing Bridge	3.0	30.9	39.3	23.4	3.4	----------	3.93	338.1
West Thumb	2.8	32.8	37.4	22.0	3.6	1.4	3.87	323.6
953:								
Fishing Bridge	5.9	21.3	30.8	35.3	6.1	.6	4.22	350.8
West Thumb	2.0	30.0	43.5	19.7	4.8	----------	3.96	331.8
954:								
Fishing Bridge	7.4	18.8	30.1	39.3	4.4	----------	4.14	339.4
West Thumb	6.2	28.5	44.0	19.3	2.0	----------	3.82	332.0
955:								
Fishing Bridge	3.0	20.1	26.9	41.3	8.7	----------	4.23	347.2
West Thumb	6.8	32.0	39.4	19.0	2.5	.3	4.06	339.0
956:								
Fishing Bridge	.8	18.4	43.0	33.6	4.2	----------	4.21	352.3
West Thumb	----------	30.5	46.2	22.3	1.0	----------	3.94	344.0
957:								
Fishing Bridge	1.4	12.3	53.5	30.8	2.0	----------	4.20	353.2
West Thumb	.7	13.5	47.4	36.3	2.1	----------	4.26	353.3
958:								
Fishing Bridge	1.0	10.5	46.3	38.1	4.0	----------	4.36	357.6
West Thumb	2.0	13.3	48.5	34.6	1.6	----------	4.28	358.0
959:								
Fishing Bridge	8.1	20.0	54.4	17.4	.1	----------	3.81	355.5
West Thumb	1.3	20.2	68.0	10.4	.1	----------	3.88	353.2
960:								
Fishing Bridge	3.2	32.4	49.5	14.6	.4	----------	3.77	345.7
West Thumb	4.1	29.7	53.7	11.1	1.4	----------	3.76	347.4
961:								
Fishing Bridge	.9	26.8	60.8	11.5	----------	----------	3.83	340.5
West Thumb	1.7	27.1	59.2	11.9	.1	----------	3.82	345.4

TABLE 8.—*Age composition of catches from Fishing Bridge and from West Thumb docks for three periods of angling season, by years, 1950 to 1959*

Year and location of catch	For period June 15 to July 11,[1] percent in age groups—			For period July 12 to August 8, percent in age groups—			For period August 9 to September 5, percent in age groups—		
	II, III	IV	V, VI, VII	II, III	IV	V, VI, VII	II, III	IV	V, VI, VII
950:									
Fishing Bridge	6.2	32.6	61.2	21.0	43.8	35.1	32.6	49.2	18.1
West Thumb	21.6	30.6	47.7	85.2	7.4	7.3	80.0	12.0	8.0
951:									
Fishing Bridge	1.7	18.1	80.1	29.5	27.4	43.1	31.7	55.3	13.0
West Thumb	30.0	45.0	25.0	41.6	39.4	19.0	31.6	43.1	25.3
952:									
Fishing Bridge	13.4	37.8	48.8	42.5	43.5	14.0	50.4	33.6	16.0
West Thumb	22.7	40.1	37.2	29.9	36.3	33.8	47.8	32.9	19.2
953:									
Fishing Bridge	31.6	42.4	26.0	25.8	37.6	36.6	39.2	41.9	18.9
West Thumb	20.4	48.0	31.6	36.6	41.6	21.8	46.7	38.3	15.0
954:									
Fishing Bridge	14.1	25.3	60.6	30.7	39.8	29.5	45.5	29.5	25.0
West Thumb	28.2	47.5	24.3	36.2	40.8	23.0	34.6	46.0	19.3
955:									
Fishing Bridge	5.5	26.0	68.5	50.4	28.8	20.7	----------	----------	----------
West Thumb	16.2	45.9	37.9	52.5	36.0	11.5	41.6	51.8	6.6
956:									
Fishing Bridge	12.3	36.9	50.8	34.5	45.4	20.0	21.7	55.0	23.3
West Thumb	26.4	49.1	24.5	31.0	44.3	24.7	50.0	36.4	13.6
957:									
Fishing Bridge	6.0	46.4	47.6	20.3	57.2	22.5	17.9	58.2	23.9
West Thumb	11.3	50.8	37.9	12.7	42.7	44.5	23.2	50.0	26.8
958:									
Fishing Bridge	4.6	45.6	49.7	32.5	46.5	20.9	18.5	50.0	31.5
West Thumb	12.0	49.1	38.9	17.3	51.8	30.9	20.0	36.0	44.0
959:									
Fishing Bridge	9.7	58.9	31.4	20.9	54.5	24.5	71.9	28.1	0.0
West Thumb	10.6	69.9	19.5	29.0	65.4	5.6	14.0	57.9	28.1

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[1] In 1950, 1951, and 1952, data were collected from May 30 to July 11.

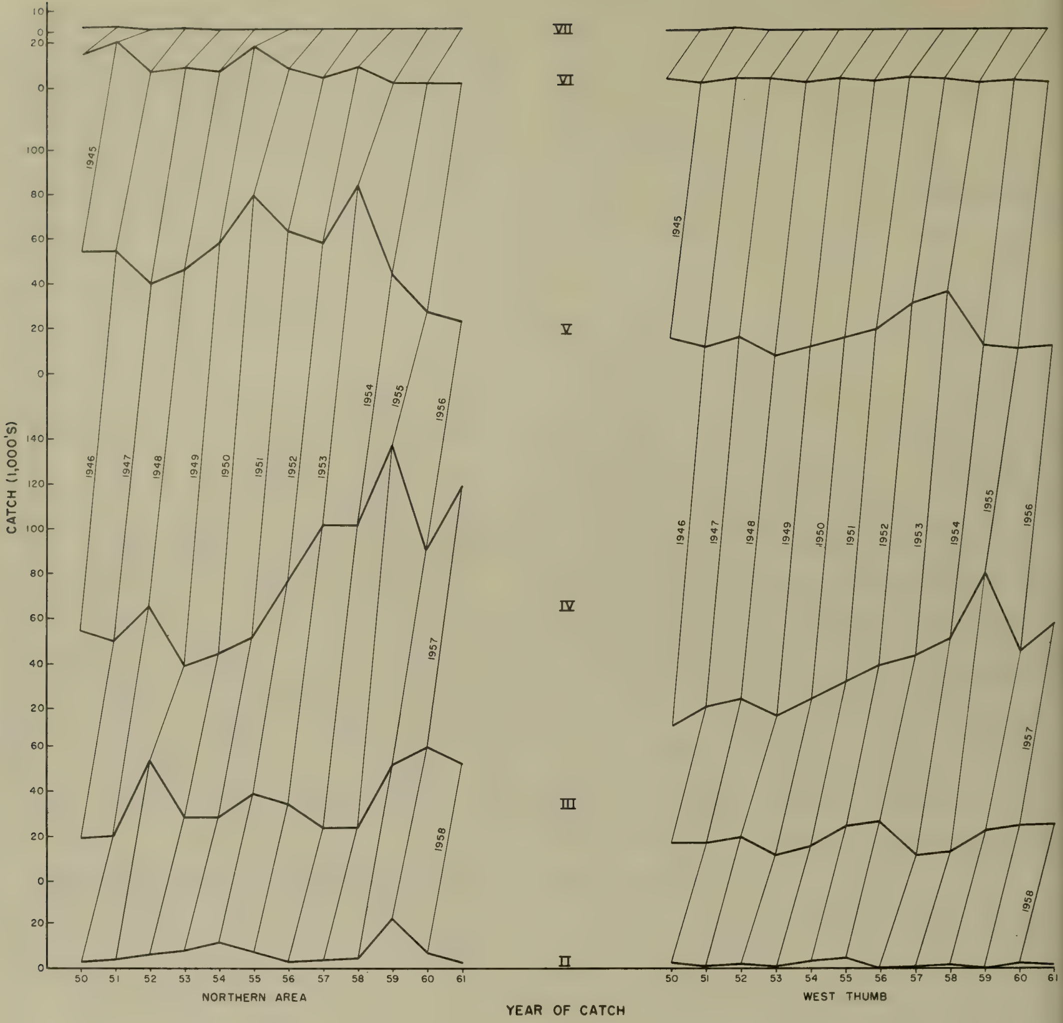

FIGURE 3.—Age composition of the northern area and West Thumb catches, 1950 to 1961. Slanting lines connect year classes.

catch. The 1953 year class contributed strongly to the catch at ages II, III, IV, and V. The 1955 year class contributed strongly at age IV, but few fish were left at age V in 1960. In both areas the general trend has been an increase in the precentage of fish of ages II, III, and IV in the catch and a decrease in fish of age groups VI and VII.

Maturity

Knowledge of the maturity of the catch is necessary for management of this fishery because the length range of the catch is small, the age composition of the catch and spawning runs are similar, and it is essential to preserve adequate spawning stocks for natural reproduction. A mature fish by our definition is one which has spawned previously, is spawning, or will spawn in the year of capture.

The maturity of the catch was determined by direct observation of fish caught before July 15, when the stage of maturity could be easily distinguished. Maturity of the entire season's catch was determined by adding a growth increment as explained in appendix B. Most of the catch is mature fish with percentages by year ranging from 64.26 to 84.76 (table 9). This condition would be expected since so much fishing is dependent upon spawning concentrations. Cutthroat trout in Yellowstone Lake become available to the fishery at only a slightly smaller size than when they enter the spawning runs. These data on maturity disagree partially with the fishing and natural mortality rates in the lake obtained from tagging of spawners by Ball and Cope (1961). They calculated the fishing mortality of postspawners from spawning streams to be a maximum of 15.2 percent, and their data indicate that the fishery is dependent almost entirely on prespawning fish. Their term "prespawners" must include immature fish, mature fish on their initial spawning migration, and fish that have spawned previously. Tagging mortality after the trout left the spawning stream was not evaluated in that study. A comparison of returns to spawning runs of 2,561 tagged and 3,928 fin-clipped spawners in Arnica Creek in 1952 and 1953 showed that tagged fish experienced an average of 171 percent higher apparent mortality than fin-clipped fish. This discrepancy would explain the low number of tagged postspawners that were recovered in the catch.

TABLE 9.—*Percentage of mature fish in the catch at Fishing Bridge and at West Thumb docks, by years, 1950 to 1961*

Year	Percent mature in catch at—		Mean percent mature
	Fishing Bridge	West Thumb docks	
1950	84.31	65.70	75.01
1951	75.71	66.89	71.30
1952	67.52	66.81	67.17
1953	74.34	64.53	69.44
1954	70.91	64.26	67.59
1955	71.93	71.76	71.85
1956	79.65	75.54	77.59
1957	84.76	78.74	81.75
1958	82.95	82.81	82.88
1959	80.39	76.88	78.64
1960	76.87	74.49	75.68
1961	71.83	73.55	72.69

CHANGES IN THE POPULATION

Measurements of growth rates, spawning runs, mortality rates, and year-class strength are available on the Yellowstone Lake cutthroat stocks from 1945 to 1961. This section discusses the fluctuations in these measurements with respect to whether they were caused by fishing or by natural fluctuations in abundance.

Growth rate

Bulkley (1961) studied growth rate of cutthroat trout from Yellowstone Lake from 1946 to 1958. These data can be briefly summarized as follows: (1) All growth-rate changes appeared to be caused by differences in stock density; (2) growth rates of fish from different parts of the lake were similar from 1953 to 1955; (3) growth rates were similar for both sexes; (4) the long-term trend showed an increase in growth to 1956 and a leveling off in 1957 and 1958; (5) the increase in rate of growth occurred only among fish of age groups IV, V, and VI, which were the age groups most heavily exploited by the fishery.

In the present study, data on growth rate from 1958 to 1960 were included, and all data were separated by area (fig. 4, app. C). The increase in growth was consistent for combined age groups with a leveling off from 1956 to 1958 followed by a drop in 1959. The northern area stock showed another increase in 1960. When growth rate is compared with data on age composition of the catch (fig. 3), size of catch (table 3), and mortality rates presented later, the reason for the drop in mean growth rate in 1959 becomes apparent. Extremely heavy exploitation of the 1955 year class at age IV occurred in 1959 which removed both slow- and fast-growing fish and almost eliminated this year class from the stock. Samples from years previous to 1959 contained a larger percentage of fast-growing fish and are not directly comparable with the 1959 sample. The very large catch in 1958 and 1959 also caused an increase in growth rate of fish of age groups II and III, particularly in the northern area.

When growth rate for combined age groups is plotted with the catches for the northern area and for West Thumb (fig. 5), it can be seen that the increase in growth rate was closely related to size of the catch. Only a decreased stock density could explain this relation. For yield considerations we have assumed that the optimum growth rate for the catchable stock in this environment was reached during the period from 1956 to 1959. Optimum growth rate by our definition means the maximum sustained growth rate that can be attained by the catchable stock in Yellowstone Lake under present environmental conditions. Growth rate of the northern area stock was higher in 1960 than this optimum level, but it is questionable whether this high rate of growth can be maintained. We have assumed that optimum growth rate was reached in 1956 because the growth rate did not continue to increase in spite of large increases in the catch from 1957 to 1959. A further decrease in stock density occurred during this period because of the recruitment of several weak year classes to the fishery, heavy exploitation of these year classes, and a reduction in size of several spawning runs.

An increase in growth rate may be undesirable if it is not accompanied by earlier age of maturity, as heavy exploitation of prespawning fish can result.

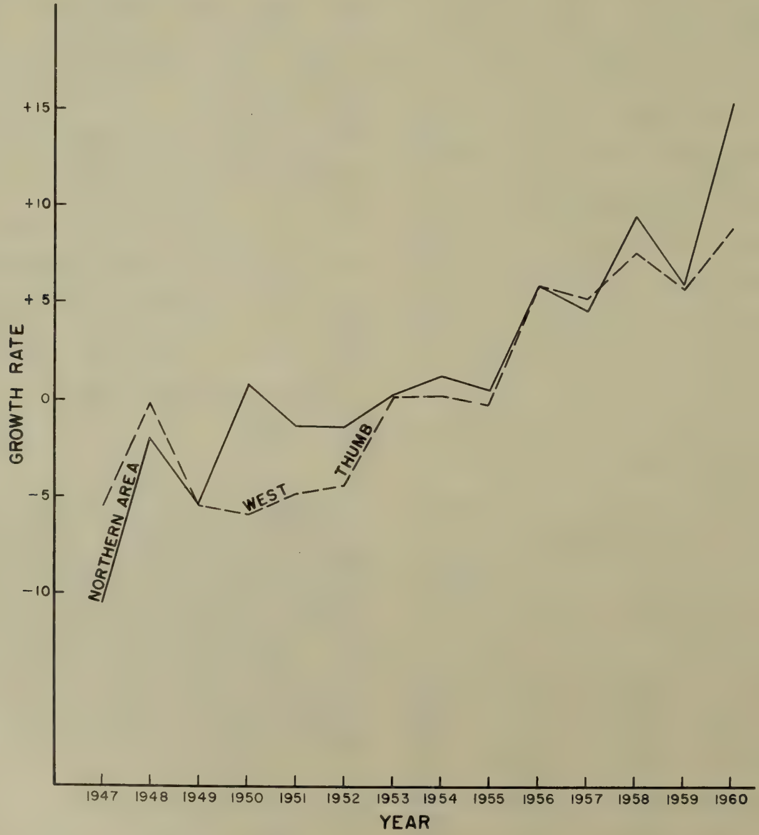

FIGURE 4.—Percentage deviation from mean growth rate by year for northern area (solid line) and West Thumb (broken line), 1947 to 1960.

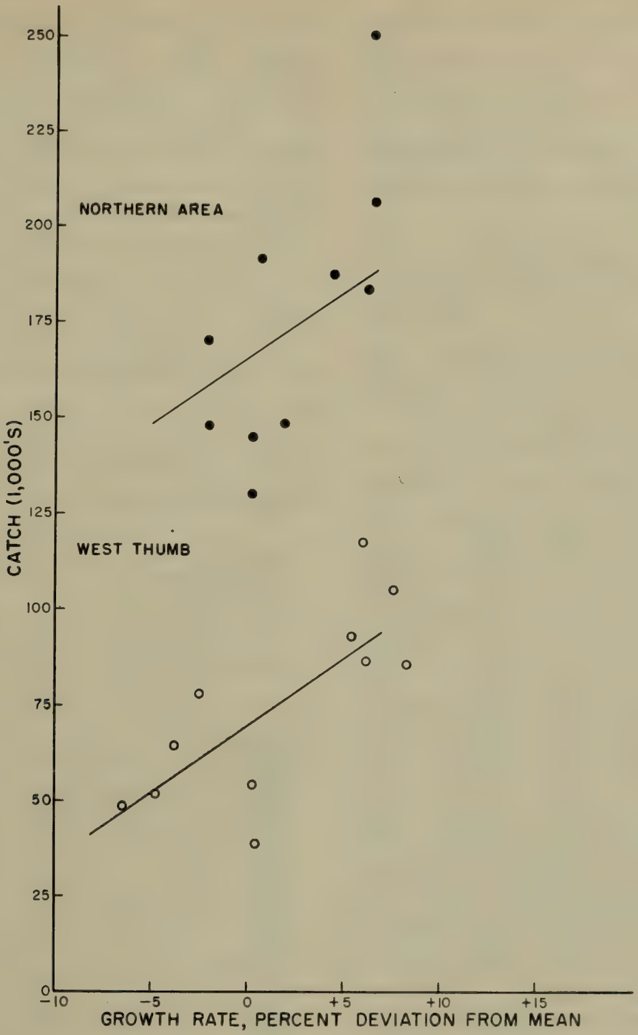

FIGURE 5.—Growth of fish from West Thumb and northern area compared with catch for same year. Growth rates expressed as percentage deviation from mean of all age groups. Regression lines calculated to show trend.

Availability by length has been rather constant in this fishery. There has been no evidence that fish are maturing at an earlier age. Earlier maturity may become more evident in the future since fish of age groups II and III showed an increased growth rate in 1958, 1959, and 1960. Later in this report we show that the increase in growth rate of these age groups did not occur until overfishing commenced, which suggests that exploitation of prespawners should not be excessive if the stock is managed at equilibrium yield.

It may be expected that the increase in growth rate in recent years would be accompanied by an increase in condition factor (K). Condition factor has been used occasionally as an index of stock density on the assumption that reduced density would allow increased growth in weight as well as length. MacGregor (1959) found that condition factor was a valuable measure of population size of the

Pacific sardine. Condition of Yellowstone Lake cutthroat varied so much with stage of maturity, sex, size, and time of year that no segment of the fishable stock or the catch could be measured adequately to determine variations in condition factor.

Spawning runs

The size of the spawning runs in six tributary streams has been measured each year since 1945, except for a few years. The sampled streams are in different areas of the lake (see fig. 1), but their relation to the total spawning population is unknown. Ball and Cope (1961) found 14 major and 26 minor tributaries to Yellowstone Lake, and the sampled streams are all major by their classification. Contributions from small tributaries to the lake stock undoubtedly fluctuate greatly because wave action often dams the mouths of small streams with sand spits, and stream flow percolates through the gravel. Both postspawners and immatures are trapped behind the sand spits as they attempt to move toward the lake, and mortality is undoubtedly high. Sand spits never block the mouths of the major tributaries, although a large lagoon at the mouth of Arnica Creek frequently becomes cut off from the lake during the late summer in low-water years.

The location of the sampled streams subjects the postspawners to different rates of fishing pressure. Ball and Cope (1961) found that the average fishing mortalities in the 1949–53 period for the five streams were as follows: Pelican, 12.2 percent; Arnica, 9.3 percent; Clear, 9.4 percent (1953 only); Grouse, 3.7 percent; and Chipmunk, 5.2 percent. Evidence presented earlier suggests that the true fishing mortalities are higher than the percentages shown above, but the ratios among streams are probably correct.

Some characteristics of all spawning runs are as follows: (1) Homing of adults is strong, since 96.8 percent of the returns from a tagged sample of 17,836 fish were later recovered in the same stream as tagged (Ball, 1955); (2) homing of immatures is present, since 19.9 percent of 644 fin-clipped trout returned to the same stream as marked and none to any other sampled stream; (3) differences in time of migration by stream or season are related to water levels and temperatures (Cope, 1956); (4) each stream has a consistent annual pattern of spawning migration; and (5) age groups IV and V include most spawners although III's were important in some years (table 10).

Reliable data on the amount of fishing pressure exerted on prespawners and their movements in the lake are not available.

TABLE 10.—*Age composition of spawning runs in five tributaries of Yellowstone Lake, by years, 1949 to 1961*

Stream and year	Percent in age group—				
	III	IV	V	VI	VII
Pelican Creek:					
1949	3.4	79.4	16.6	0.6	0
1950	4.6	59.6	30.3	4.3	1.2
1951	2.5	55.4	38.1	4.0	0
1952	14.3	60.3	22.0	2.7	.7
1953	8.3	62.8	25.6	2.6	.6
1954	5.6	55.6	35.2	3.7	0
1955	6.6	57.9	32.0	3.4	.1
1956	6.2	60.6	31.7	1.5	0
1957	2.9	73.2	23.6	.3	0
1958	3.0	65.0	32.0	0	0
1959	2.1	71.2	23.3	3.4	0
1960	4.7	60.6	31.8	2.5	.3
1961	7.1	54.5	36.9	1.5	0
Arnica Creek:					
1951	11.4	45.3	32.2	10.4	.7
1952	16.6	49.3	32.5	1.6	0
1953	11.1	58.9	29.0	1.0	0
1954	11.5	37.5	47.5	3.5	0
1955	8.4	61.5	28.8	1.3	0
1956	9.8	55.8	31.9	2.5	0
1957	2.9	41.0	53.7	2.4	0
1958	1.9	30.7	59.9	6.5	1.0
1959	4.3	62.6	32.2	.9	0
1960	17.5	66.1	13.4	3.0	0
1961	9.2	66.7	23.2	.9	0
Chipmunk Creek:					
1949	1.8	41.0	47.7	6.3	3.2
1950	.4	38.0	48.6	12.0	1.0
1951	.2	39.2	45.8	12.9	1.9
1952	1.9	46.1	38.6	12.2	1.2
1953	.7	37.0	36.5	23.2	2.5
1954	.9	31.8	40.9	20.6	5.7
1955	2.7	66.8	24.9	4.8	.8
1956	2.5	44.1	40.1	11.9	1.4
1957	.4	43.6	48.6	6.3	1.1
1958	.2	36.4	56.0	6.9	.5
1959	1.0	34.8	48.4	5.8	0
1960	.5	47.7	48.8	2.7	.3
1961	1.3	44.8	49.9	3.2	.8
Grouse Creek:					
1952	0	55.2	43.4	1.4	0
1953	4.0	63.4	28.9	3.3	0
1954	0	24.4	59.4	13.5	2.7
1955	0	50.6	42.8	6.6	0
Clear Creek:					
1953	0	60.7	35.2	4.1	0
1954	0	58.3	33.3	7.8	.6
1955	5.3	75.9	18.8	0	0

We expect, from data on homing, that prespawners produced in a stream near an area of heavy fishing pressure will be subjected to a greater rate of exploitation than those produced from a stream in an area of low fishing pressure. Accordingly, the prespawners from Pelican Creek are subjected to greater fishing pressure than those from Chipmunk Creek. It is probable that the differences in fishing mortalities of postspawners for the five sampled streams presented above could be applied, in general, to prespawners. Characteristics of each spawning stream were described by Ball and Cope (1961), and the differences in the spawning stocks among streams are discussed below.

Arnica Creek

Arnica Creek, one of the smallest streams studied, had spawning runs ranging from 1,755 in 1959 to 6,200 in 1954 (table 11). Spawning migrations were spread over a period of about 30 days, and large runs showed migration patterns similar to those of small runs. Age groups IV and V were most important, but III's were common from 1951 to 1954 (table 10). Bulkley (1961) found that strong year classes were produced in 1949, 1951, and 1953, and weak year classes in 1948 and 1955. No regular cyclical patterns of year-class strength were noted. A clear understanding of Arnica Creek year-class strength is obscured because the large lagoon at the mouth is occasionally cut off from the main lake in August; this factor probably influences the survival rate of immatures leaving the stream. In 1960 and 1961 it was observed that the outlet of Arnica lagoon was cut off from the lake in the spring, and this prevented spawners that had wintered in the lake from entering Arnica Creek until after a significant part of the spawning run had migrated upstream. The early part of the run must have been composed of spawners that wintered in the lagoon. This lagoon has probably been cut off in previous years and has influenced mortality rates of both adults and immatures.

It is not possible to relate the effects of increased catch to Arnica Creek spawning runs precisely, but the low runs in 1959, 1960, and 1961 could be attributed only to the heavy catches in West Thumb. Effects of fishing were strong in the 1960 run as shown by the few fish of age group V present.

TABLE 11.—*Annual counts of spawners in six tributaries of Yellowstone Lake, 1945 to 1961*

Year	Pelican Creek	Clear Creek	Chipmunk Creek	Grouse Creek	Cub Creek	Arnica Creek
1945	22, 297	13, 123	13, 589	13, 264	6, 864	--------
1946	14, 588	16, 429	22, 496	6, 171	11, 027	--------
1947	19, 613	23, 211	11, 107	8, 158	11, 344	--------
1949	32, 431	7, 274	13, 580	11, 790	--------	--------
1950	15, 076	10, 459	12, 255	9, 635	2, 179	3, 881
1951	9, 423	16, 879	12, 585	12, 188	2, 676	4, 755
1952	7, 953	10, 269	9, 989	6, 949	2, 056	3, 523
1953	12, 413	10, 323	7, 836	7, 441	3, 848	4, 524
1954	10, 340	3, 161	5, 371	3, 756	3, 446	6, 200
1955	12, 400	7, 929	3, 880	--------	2, 558	4, 700
1956	--------	--------	--------	--------	--------	3, 912
1957	17, 500	8, 398	9, 605	--------	2, 976	3, 812
1958	7, 259	9, 355	8, 922	13, 462	1, 747	4, 041
1959	22, 198	3, 353	6, 225	7, 330	7, 257	1, 755
1960	3, 363	--------	3, 456	10, 570	--------	2, 346
1961	15, 568	8, 119	4, 382	4, 235	5, 087	2, 090

Pelican Creek

Pelican Creek has the largest spawning run of the streams sampled, and its postspawners are subjected to the greatest fishing pressure. The number of spawners ranged from 3,363 in 1960 to 32,431 in 1949. Strong year classes were produced in 1949, 1953, 1955, and 1958. Weak year classes were produced in 1948, 1952, and 1956. Bulkley and Benson (1962) found a high positive correlation between strong year classes in Pelican Creek and low water levels, and developed a formula for prediction of year-class strength based on water levels during the period of spawning and incubation. With the great importance of stream water levels to year-class strength, a close relation between size of escapement and year-class strength cannot be expected.

The minimum escapement necessary for Pelican Creek varies annually with environmental conditions, which emphasizes the danger of maintaining escapement at a low level. A small escapement may be able to produce an average year class if environmental conditions are favorable, whereas the same amount of escapement in a year of unfavorable conditions may produce practically no progeny. To assure adequate reproduction, Pelican Creek spawning runs should not go below 7,000 fish, and should be above 10,000 fish. The small 1960 spawning run of 3,363 fish was attributed to the very heavy catch in 1959 and is considered to be much too low to assure the production of a strong year class.

Clear and Cub Creeks

The spawning migrations in Clear and Cub Creeks are delayed more than those in the other streams studied because of low stream water temperatures. This condition allows heavier exploitation of prespawners during the first few weeks of each angling season because the fish are concentrated in the lake around the stream outlets.

There are not enough data to relate catch to size of spawning runs accurately for these streams. The runs in Clear Creek have been low since 1952 (table 11). The Cub Creek spawning runs were low from 1950 to 1958, but a large number of trout were counted in 1959. The trend in both of these streams has been downward, and it is undoubtedly related to increased catch.

Chipmunk and Grouse Creeks

Chipmunk and Grouse Creeks are in the south end of the lake where fishing pressure is light, although most recoveries of tagged postspawners were in West Thumb, with a few in the northern area. Both these streams have an unusually high percentage of fish of age group V in each spawning run (table 10), and Bulkley (1961) suggested that recruitment into spawning runs may not be completed until age V. Thus, any increase in the catch of age group IV fish (see fig. 3) would influence these spawning runs to a greater degree than the other study streams. The trend in Chipmunk has been downward since 1952 (table 11). Bulkley and Benson (1962) related year-class strength in Chipmunk to various environmental factors. Good production can be expected if water levels are low and relatively stable during the incubation period, and if spawner numbers are adequate. An escapement of 4,600 fish appears to be suitable.

Weak year classes are produced in years when lake water levels are high or fluctuate widely during June and July, even when spawning runs are large. The low spawning run in 1960 resulted from an overexploited 1955 year class and a weak 1956 year class. The 1955 year class was abundant because environmental conditions were ideal for high production in 1955 and the escapement was not too low, but the large catch of age group IV fish in 1959 caused overexploitation of this year class before many of the fish reached maturity. Grouse Creek data are scanty, and no definite conclusions can be made.

Mortality rates

Mortality rates for Yellowstone Lake cutthroat have been determined from tagging studies and from catch curves. Ball and Cope (1961) calculated mortality rates from returns of tagged spawners in Yellowstone Lake from 1949 to 1953. Their figures on fishing mortality rates of postspawners averaged 8.2 percent and ranged from 2.2 to 15.2 percent. Their data indicate that most of the catch must come from prespawners. They did not consider the differential mortality between tagged and untagged fish in the lake, as explained earlier, and the average of 8.2 percent fishing mortality was probably low.

Catch curves are obtained by plotting the $\log_{10}$ frequency of occurrence of fish in the catch against age. Total instantaneous mortality rate can then be obtained by determining the natural logarithm of the difference in number of fish caught from two consecutive age groups. A catch curve from 1 year's data is only valid for computing mortality and survival rates if year-class strength remains relatively stable from year to year. This assumption could not be met in Yellowstone Lake, as wide fluctuations in year-class strength occur. Catch curves of individual year classes are difficult to interpret because contributions of a year class to the catch at different ages is influenced both by the size of the catch in different years and by strength of neighboring year classes. We have used year-class catch curves to calculate total instantaneous mortality rates. These should not be interpreted as actual values, as both catch and year-class strength varied annually in the fishery. They have been included only to illustrate the changes which occurred between the times the 1946 and 1956 year classes entered the catch.

Mortality rates of fish in age groups IV and V were computed from catch data for West Thumb and the northern area presented in appendix E. Mortalities of age groups II and III were not calculated owing to incomplete recruitment of these age groups to the fishery. The catch-curve data and resultant mortality rates for the northern area are a more reliable index of stock conditions than those from West Thumb. The West Thumb stock is composed of fish from many spawning streams which differ considerably from each other in year-class strength and in seasonal recruitment to the fishery. Hence in any given year the catch may have a different proportion of fish from a particular stream than in the previous or ensuing years. The northern area stock is derived principally from the Yellowstone River and Pelican Creek, which are similar in relative year-class strength and in seasonal recruitment to the fishery.

For the northern area, mortality rates of age group IV fish were grouped rather close together and remained below 0.5 until the catch exceeded 200,000 fish in 1958 (tables 3, 12). Mortalities at this age for later year classes varied from 0.845 to 1.637. Mortality rates of age group V remained very close to 2.0 prior to the 1952 year class but with much less fluctuation than age group IV. Rate for the 1953 year class jumped to 5.812 with the 1958 catch and remained high for the 1954 year class also.

TABLE 12.—*Instantaneous total mortality in cutthroat trout of age groups IV and V from northern area and from West Thumb, by year classes, 1946 to 1956*

Year class	Northern area		West Thumb	
	Age IV	Age V	Age IV	Age V
1946	0.017	2.228	0.059	1.574
1947	.233	1.618	.411	2.044
1948	.381	1.948	1.139	1.960
1949	(1)	1.251	.488	1.667
1950	(1)	2.332	.463	2.845
1951	(1)	2.798	.470	2.299
1952	.310	1.886	.177	2.990
1953	.184	5.812	.183	5.741
1954	.845	4.096	1.432	2.314
1955	1.637	(2)	2.123	4.589
1956	1.402	----------	1.385	----------

[1] Mortality not calculated owing to recognized sampling error. More V's than IV's were sampled from the catch.

[2] No age group VI fish in sample of 1961 catch.

In West Thumb, total instantaneous mortality of age group IV did not exceed 0.5, with the exception of the 1948 year class, until the catch exceeded 100,000 fish in 1958. From 1958 to 1960 the mortality rate for age group IV remained well above 1.0 even though the West Thumb catch dipped to 85,953 fish in 1960. Mortality rates for age group V fish climbed slowly and somewhat erratically until that of the 1953 year class, which was almost double the mortality of the previous year class with a value of 5.741. The 1954 year class was not adversely influenced according to these data, but the 1955 year class again experienced high mortality at age V.

There is a possibility that natural mortality could have varied enough to influence the total instantaneous mortality rate, which comprises both natural and fishing mortality. The major source of natural mortality for the catchable-sized stock is that associated with spawning. Ball and Cope (1961) determined stream mortality for five spawning streams through tagging from 1949 to 1953. Their estimates from all streams ranged from 23.0 to 94.9 percent and averaged 48.1 percent. This range gives the impression that spawning mortality varies greatly among streams and among years. A close examination of the tag returns shows that the large variations among years occurred only when tag returns were low, and several sources of error could have influenced the percentages, as mentioned by the authors. The data from 1951 to 1953 for Arnica and Pelican Creeks, when the percentages of tag returns were the highest, did not show these extreme variations since mortality rates ranged only 15 percent for Arnica and 9 percent for Pelican. The Arnica spawning mortality in 1953 is known to be accurate because it agreed closely with a direct count. Thus, the hypothesis that spawning mortality in any single stream is fairly constant agrees closely with the above mortality data when only the most reliable tagging data are considered. The high total mortality rates for the 1953 to 1955 year classes must have been due to increased catch since no other recognized variable was present during the years when these year classes were being exploited. The consistently low instantaneous mortality rates among year classes 1946 to 1952 support the conclusion that increased fishing mortality caused the changes observed in the later year classes.

Year-class strength of catch

Bulkley (1961) analyzed the relative year-class strength of the northern area and West Thumb catches, utilizing the method of el-Zarka, (1959) which is based upon the percentages of all age groups from each year class that enter the catch. A summary of the data used for computing relative year-class strength from 1944 to 1957 is presented in appendix D. In the northern area, strong year classes occurred in 1945, 1949, 1953, and 1957, or at 4-year intervals (fig. 6). In West Thumb, strong year classes were produced in 1947, 1952, 1953, 1955, and 1957. A large annual variation in year-class strength in both areas was especially noticeable. The West Thumb stock varied more irregularly than the northern area because it is derived from many spawning streams which vary independently in year-class strength from year to year.

There is no indication that the increased catch from 1950 to 1961 adversely influenced year-class strength, although it definitely reduced the size of spawning runs entering most of the study streams. The 1960 spawning run of 3,363 fish in Pelican Creek was probably closest to the point where escapement became the limiting factor in production for that stream. Additional data will be needed for each spawning stream to ascertain the level of escapement at which environmental conditions are no longer the major limiting factor in year-class production, although this relation obviously varies annually in the same stream as well as among streams.

The increased catch introduced a bias into the estimates of relative strength of the 1954 and 1955 year classes. This bias appears in the el-Zarka method whenever the catch is sufficient to reduce the size of older age groups in the population and shifts to younger age groups. The 1954 year class is probably represented in figure 6 as being slightly weaker than it actually was, and the 1955 year class is definitely represented as being too weak.

MAXIMUM EQUILIBRIUM YIELD

Method

Maximum equilibrium yield has been defined by Ricker (1958a) as the largest average catch which can continuously be taken from a stock under current environmental conditions. Overfishing com-

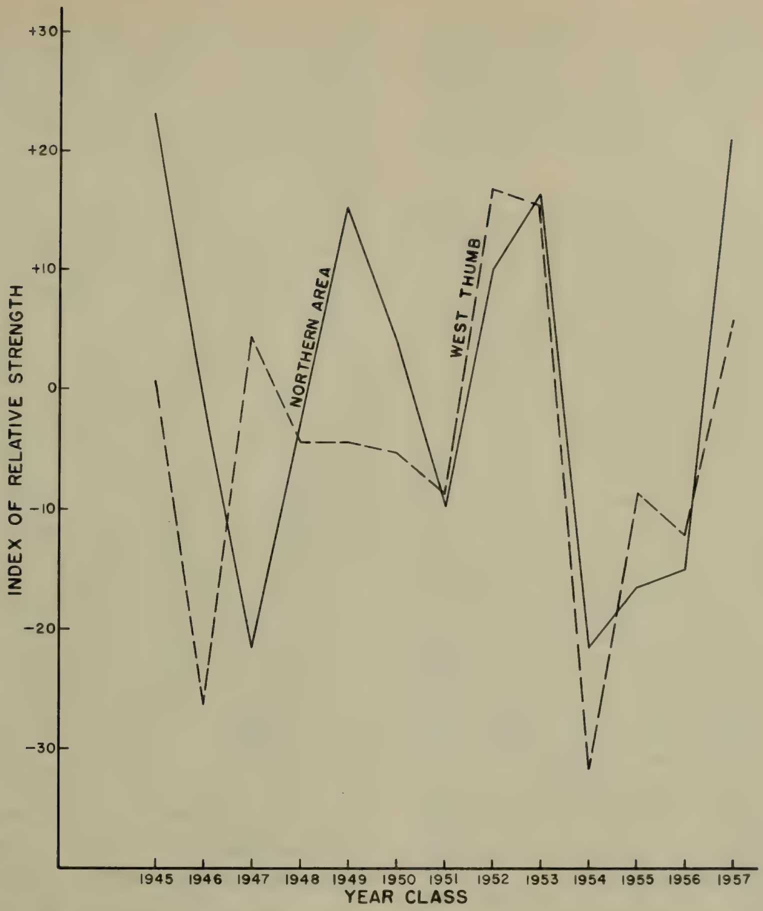

FIGURE 6.—Relative year-class strength based on contributions to the catch of all age groups for northern area (solid line) and West Thumb (broken line), 1945 to 1957.

mences whenever the catch surpasses equilibrium yield. For Yellowstone Lake, yield is considered numerically because it can be administered most easily on this basis. We have defined overfishing in this stock as the removal of so many fish that the following conditions exist: (1) Growth rate remains high, but the mean size and mean age of the catch decrease to a greater extent than can be attributed to variations in year-class strength; and (2) the spawning stock is excessively reduced by the catch. This definition of overfishing follows the more generalized definition of Rounsefell and Everhart (1954) with the addition of methods for its recognition in the Yellowstone stock.

Miller (1949) studied commercially exploited whitefish populations in Alberta under various rates of exploitation and observed overexploitation of the stock. He was able to determine the conditions that preceded and ultimately caused overfishing. Overfishing resulted from overexploitation of the old age groups which caused an overabundance and slow growth of young age groups. In his words, "Overfishing begins when decreasing average age overtakes increasing growth rate and before decreased yields are evident". In that fishery overfishing was evident (but not recognized) about 2 years before collapse.

Nikolsky (1953) summarized some indices for estimating the best rate of exploitation of fish stocks and considered maximum or optimum rate of growth for the species in the environment a desirable goal.

Ricker's (1958a) explanation of the relations of reproduction curves and yield has been useful. Several ideas of Beverton and Holt (1957) were considered, although their methods could not be directly applied. Graham's (1935) ideas of surplus stock and yield were also helpful. The use of a mathematical model to express the stock changes was attempted, but insufficient data were available to construct a model without a large inherent error. Several limitations of applying mathematical models to sport fishery stocks were aptly discussed by Watt (1959). He analyzed a smallmouth bass population by several of the accepted methods and concluded that, "The form of equations for use in predicting and managing complex biological phenomena must be dictated by the ultimate reality for the phenomenon, the data themselves, not by *a priori* deductions."

Our method for estimating yield consists in establishing three stages of exploitation from our knowledge of changes that have occurred in the stock. Equilibrium yield was estimated by fitting catch data to these stages. The method requires long-term data and provides only a rough estimate of yield, but it does not require the voluminous data required for the development of an accurate mathematical model (see Watt, 1959, p. 391). Several of the criteria used in this method can be applied to many sport fishery populations.

During the first stage increased catch reduces stock density, and growth rate increases to its optimum level for the environment. Mean length of the catch remains stable, but mean age decreases. Utilization of fish foods becomes more efficient. Total instantaneous mortality rates of age group IV trout, as determined from year-class catch curves, do not exceed 0.5. Adequate spawning stocks are maintained. The most efficient production for angling purposes is attained at the maximum catch of this stage and is the maximum equilibrium yield.

In the second stage overfishing commences. Decreasing mean age of the catch overtakes increasing growth rate to a greater extent than can be attributed to variations in year-class strength. Lower mean age of the catch and lower mean lengths result. Fish of age groups II and III enter the catch prior to the time they normally do, and the number of age group IV's greatly exceeds the number of V's. The number of fish caught will remain similar to the first stage for a time, but continued removal of older age groups will lower the total catch near the end of the stage. Total instantaneous mortality rates of age group IV trout, as determined by year-class catch curves, exceed 0.5. Older groups are exploited heavily enough to reduce spawning stocks significantly, and spawning runs show a lower mean age.

The third stage will be reached when the catch removes enough of the sexually mature fish to prevent full utilization of the suitable spawning and rearing sites. Production of immatures will be reduced. Recovery from this stage will require several years or until the necessary numbers of spawners to utilize the available spawning facilities are restored to the stock.

The estimation of yield by these stages follows Ricker's use of reproduction curves to calculate yield but also considers growth rate, lengths, and age distribution of the catch. It was not possible to calculate a reproduction curve for the lake stock because there are not sufficient data to relate size of escapement to recruitment. Data from Pelican Creek show that water levels alone accounted for 94 percent of the variation in year-class strength, and

Bulkley and Benson (1962) concluded that environmental factors had such a strong influence on production that an adequate escapement could not produce a strong year class unless water conditions were favorable. As suggested earlier, the spawning run in 1960 for Pelican Creek is too low for adequate seeding, but the minimum level of escapement necessary to produce a good year class varies with environmental conditions. There is, obviously, a low limit of desirable escapement for each stream which will insure some recruitment even with adverse stream conditions. The low escapement in several streams that resulted from the increased catch during 1958 and 1959 is not a safe condition to insure adequate annual recruitment.

Calculation of estimated equilibrium yield and analyses of changes in the Yellowstone stock in relation to the above stages of exploitation are discussed below.

Calculation of yield

Northern area and West Thumb stocks will be discussed separately, and a yield for the entire lake will be considered later. Changes in stock condition as related to total catch, growth rate, mean age and length, and annual catch curves are summarized in figure 7. Annual catch curves illustrate the degree of exploitation when analyzed in conjunction with the other data, including year-class strength.

In the northern area catch, all fluctuations in mean age and length before 1958 can be attributed to differences in year-class strength and to the increase in growth rate. As explained earlier, optimum growth rate was reached in 1956. The drop in mean length and age in 1952 was due to a large number of fish of age group III in the catch from the strong 1949 year class. The increase in mean length from 1955 to 1958 was due to the heavy catch of age groups IV and V and to the increase in growth rate. Mean age changed little from 1955 to 1957, but both mean age and mean length have dropped consistently from 1958 to 1961.

Catch curves before 1959 were flat on top because age groups IV and V were caught in approximately equal numbers. Some fluctuations among age groups III, IV, and V were evident, owing to variations in year-class strength, but the same general pattern was maintained. The 1957 catch curve was peaked because fish of age group IV from the strong 1953 year class were caught in large numbers. The 1958 catch curve does not look much different from those of previous years, but both the IV's from the weak 1954 year class and the V's from the strong 1953 year class were exploited heavily, so that few V's from the 1954 class and VI's from the 1953 year class were caught in 1959. Continued heavy catches in 1959 and 1960 of the IV's and V's resulted in catch curves for 1960 and 1961 that showed exceptionally heavy exploitation. We consider an annual catch curve with a steep right slope after age IV to be indicative of heavy exploitation during the previous year as long as the steepness is not the result of variation in year-class strength. In 1959, 1960, and 1961 there were many age group III's in the catch. This exploitation caused a low mean age in 1959, 1960, and 1961, and a drop in mean lengths in 1960 and 1961. The lower mean ages and lengths in 1960 and 1961 were not caused by year-class strength but by overexploitation of the 1955 and 1956 year classes in 1959 and heavy exploitation of the 1957 and 1958 year classes in 1960. The excessive exploitation reduced the 1960 spawning run in Pelican Creek and caused overfishing. Overfishing commenced somewhere between the 1957 catch of 187,462 and the 1958 catch of 219,036.

In the West Thumb stock, age groups III, IV, and V were more equally represented in the annual catch curves before

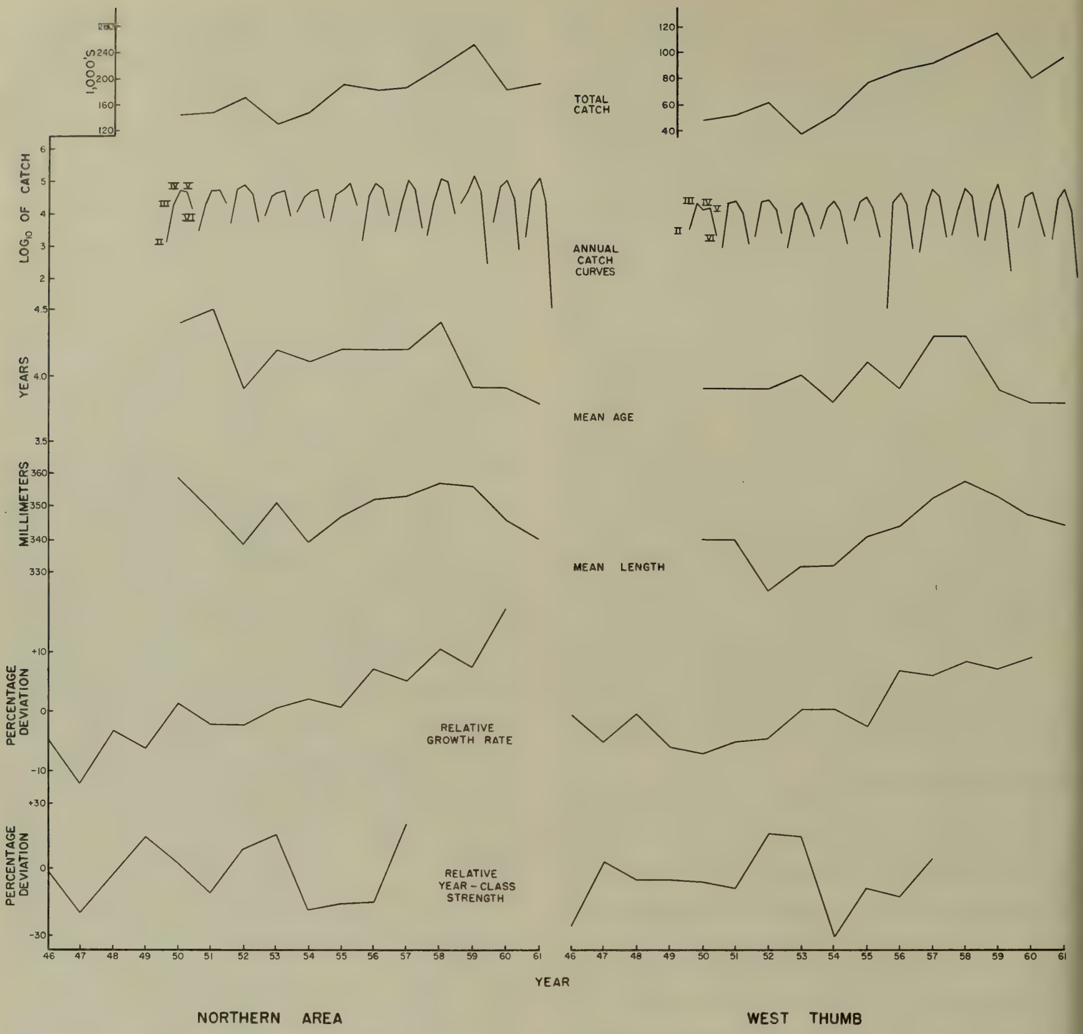

FIGURE 7.—Data from northern area and West Thumb collected from 1950 to 1961, including relative year-class strength, total catch, relative growth rate, mean lengths, mean age, and annual catch curves.

1958 because the fishery is not heavily concentrated on a spawing population. The changes in mean lengths and mean ages before 1958 can all be explained by variation in year-class strength which altered ratios of age groups in the catch. Since 1958 the mean length and mean age of the catch have dropped each year as the result of heavy exploitation. Low mean lengths in 1952, 1953, and 1954 were due to a large number of III's and few V's in the catch. The increase in mean length from 1954 to 1958 was due to the large number of IV's and V's in the catch and to increased growth rate. The mean age dropped in 1956 owing to the large number of III's from the strong 1953 year class. In 1958 the V's from the 1953 year class and IV's from the 1954 year class were heavily exploited, and there was an abrupt drop in the catch of these year classes in 1959. The heavy 1959 catch was concentrated on the IV's from the 1955 year class, and there were few V's caught from this year class in 1960. As in the northern area, West

Thumb annual catch curves had a steep slope from age group IV to age group VI after the 1958 catch. Both the Arnica Creek and Chipmunk Creek spawning runs were reduced by the 1959 and 1960 catch. The West Thumb stock was overfished in 1958 with a catch of 105,000 fish.

Catches and stages of exploitation of of the lake population are summarized as follows:

Year	Catch in—		Stage of exploitation
	Northern area	West Thumb	
1957	187, 462	92, 174	Stage one, no overexploitation.
1958	219, 036	105, 155	Heavy exploitation of IV's of 1954 year class and V's of the 1953 year class caused stock to enter stage two.
1959	250, 310	117, 171	Heavy exploitation of IV's from 1955 year class and V's from 1954 year class. Spawning runs in 1960 had fewer V's than expected. Mean length and age lower than could be attributed to year-class strength.
1960	181, 588	85, 953	Mean age and length continue to drop owing to heavier than normal catch of III's and few V's and VI's. Stock in stage two with some evidence of stage three from spawning runs as a result of the large 1959 catch.
1961	192, 211	97, 114	Mean age and length continue to drop owing to heavy catch of III's and IV's and few VI's. Stock in stage two, but some evidence of recovery in Pelican Creek spawning runs due to lighter catch in 1960.

Overfishing in both the northern area and West Thumb commenced at an unknown catch level in 1958 between the 1957 and the 1958 catch. We know that the year classes in the 1957 catch were stronger than those in the 1958 catch, but we cannot determine when the equilibrium was surpassed in 1958. Our best estimate is the average between the 1957 and 1958 catch, or about 203,000 trout for the northern area and 98,000 trout for the West Thumb area. With the addition of the southern area, the maximum equilibrium yield for the lake is estimated to be 325,000 fish with the present distribution of fishing pressure.

Since each annual catch curve may have six different year classes of various strengths, maximum equilibrium yield can be expected to vary from about 290,000 to 340,000 fish. The lowest equilibrium yield will occur when age groups III, IV, and V, the principal age groups in the catch, come from three consecutive weak year classes, and the highest yield will be possible when these three age groups come from three strong year classes. Since year-class strength varies so greatly among different streams, it is doubtful whether either extreme could occur.

The catch data in appendix E give some information on the approximate numbers of trout that were caught from year classes of different strengths by age group. It is realized that this information is susceptible to large error since differences in total catch and strength of the neighboring year classes have materially influenced these figures. It would require many years of relatively constant catch to determine the exact influence of year-class strength on the entry of age groups into the catch and resultant differences in natural mortality. From the available catch-curve data the hypothetical number of fish, in thousands, that could be caught in the northern area, without overfishing, has been estimated for weak, average, and strong year classes (table 13). Each annual catch curve contains five or more year classes of various strengths, and the shape of the annual catch curves will oscillate, even with an equilibrium catch. Overfishing can be recognized only if strength of each year class in the catch curve is considered.

TABLE 13.—*Hypothetical equilibrium yields of cutthroat trout for northern area from weak, average, and strong year classes*

[In thousands of fish]

Age group	Yield if year class is—		
	Weak	Average	Strong
II	2	5	8
III	25	30	35
IV	80	90	100
V	60	70	80
VI	6	8	10
Total	173	203	233

These overfishing conditions described for Yellowstone Lake agree quite closely with those described by Russell (1942), who discussed the overexploitation of ground fish in the North Sea. In his words,

> We have already seen in a general way what happens in a well-fished stock after the accumulation of old slow-growing fish has been swept away. The density (in terms of weight) falls gradually with increased fishing, the percentage of large fish diminishes, and finally the total yield falls, and we get overfishing.

MANAGEMENT

Regulations

Knowledge of the approximate equilibrium yield of a fish population is of little value for management unless the fishery can be regulated to attain and maintain that yield. In some populations, decreased density acts as a natural brake on stock depletion by reducing availability. Angling automatically becomes less efficient, and catch per unit of effort decreases. On Yellowstone Lake the catch per man-hour did not change much even when density of the stock was reduced and the stock overexploited, because the fishery was centered over spawning concentrations and more efficient angling gear came into general use. With a high catch per hour, a decline in fishing interest cannot be expected to slow down depletion of the stock. Additional regulations must be adopted to restrict the catch to equilibrium yield. Several possible regulations are considered here with the following questions in mind: (1) Is this regulation practical for Yellowstone Lake? (2) Will it be effective in reducing the catch without destroying the incentive to fish? (3) What will be its influence, and can its effect be predicted?

Control by quotas

An attempt can be made to manage a fishery on equilibrium yield by use of an annually adjusted number of fishing trips, or a "quota" system. Such a system will always be difficult to apply to a sport fishery because angling success and pressure are too unpredictable. For Yellowstone Lake, we do have adequate background biological data for reliable prediction within reasonable limits, and it would be possible to establish the necessary number of fishing trips to attain a desired yield; but other factors make a quota system based on fishing trips impractical for Yellowstone Lake, as well as for most sport fisheries on public waters. There would be too many administrative problems in deciding who may fish, and when, to consider such a method. Ricker (1958b) stated that the objectives of a quota system can be obtained by other methods of regulation. Catch goals by numbers or weight in commercially exploited stocks are often too difficult to administer, and regulation by season, area of fishing, and the like, are frequently used to attain equilibrium yield. Some of these methods are applicable to the Yellowstone Lake fishery.

Shortening of fishing season

Shortening the fishing season is another method to be considered for restricting the catch. The present June 15–October 15 fishing season on Yellowstone Lake is already short, and fishing pressure is very light after the Labor Day weekend (around September 5) when most of the tourist accommodations are closed. Fishing is essentially limited to a 2¾-month period. As shown in table 5, 60 percent of the catch is made before August 1. Closure of the season at a date earlier than October 15 would not be effective for preventing overexploitation unless the season closed August 15 or earlier. This early closing date would stop fishing when the fish are in the best condition and furnish the most sport.

A later opening day such as July 1 would reduce the catch enough to be of some biological value. The fishing from June 15 to June 30 is concentrated on spawning fish, and some of these fish would disperse enough, before a July 1 opening, to lessen their chances for capture. The present opening date protects much of the spawning, but a July 1 opening date on the lake and river would have considerable value, especially in years of low escapement or delayed spawning. In 1960, 33.4 percent of the June 15–30 catch landed at Fishing Bridge dock consisted of ripe or partially spent fish. It is not possible to regulate the opening of any fishery precisely enough by chronological time to allow all fish to spawn before capture because "biological time" varies too much among years. Even vast amounts of past data on timing of spawning migrations may not give the necessary information for effectively regulating the catch during any specific year. A later opening would have an undesirable economic influence on the boat concessionaires because it would mean no fishing when fish are most available. The present fishing season should be retained until other methods of reducing the catch have been tried.

Minimum size limits

Size limits are used to protect breeding stocks and/or to adjust the size distribution of the catch to attain the most desirable yield (Allen, 1954). For Yellowstone Lake a minimum size of 12 inches (305 mm.) is recommended. The effects of this size limit on the catch and on various age groups from 1950 to 1961 are shown in table 14. Up to 1955, the catch would theoretically have been reduced by an average of 12.7 percent with this size limit. Since 1956, when the growth rate reached its optimum, this size limit would have reduced the total catch by a maximum of 13.9 percent and an average of 9.5 percent. Obviously the actual reductions on size of catch would not approach these percentages, as many fishermen would continue fishing until they did catch fish large enough to keep.

With the present growth rate, a 12-inch size limit would essentially eliminate age group II from the catch, reduce the catch of age group III by about one-half, and affect virtually no older age groups. It would allow higher survival of prespawners, but it would not have a great influence on the average size of the fish in the creel (see table 6). A lower size limit would have little value because of the low availability of small fish. A larger size limit of 13 inches would almost eliminate age group III from the catch, which is not considered necessary at this time. The increase in the catch of age groups II and III in 1959 and 1960 suggested that some

TABLE 14.—*Percentage of fish less than 12 inches long in total catches and in age groups of catches from the northern area and from West Thumb, by years, 1950 to 1961*

[All fish of age group V and older are above 12 inches]

Year	Percent in total catch from—			Percent in age group—					
				II		III		IV	
	Northern area	West Thumb	Northern area and West Thumb	From northern area	From West Thumb	From northern area	From West Thumb	From northern area	From West Thumb
1950	5.7	16.1	8.3	100.0	100.0	50.0	74.2	1.1	2.1
1951	13.3	15.0	13.7	100.0	100.0	63.3	53.0	3.2	3.7
1952	13.3	15.8	13.9	92.6	90.0	42.2	56.5	0	5.8
1953	13.0	12.0	12.8	95.0	90.9	53.5	45.0	.9	3.3
1954	12.3	18.8	14.0	95.4	96.6	41.7	50.0	0	3.3
1955	14.1	10.9	13.2	90.0	84.2	61.6	30.4	2.5	.6
1956	6.5	7.3	6.8	100.0	------------	33.3	33.3	3.1	0
1957	4.8	8.4	6.0	100.0	100.0	49.4	67.5	0	0
1958	8.9	7.6	8.5	85.9	95.0	47.5	47.2	0	1.3
1959	8.8	11.3	9.6	69.6	100.0	46.3	40.3	0	4.2
1960	9.3	9.3	9.3	100.0	100.0	49.1	50.8	0	1.6
1961	15.4	12.6	13.9	100.0	100.0	52.0	39.9	1.0	1.5

measure is necessary to reduce the exploitation of these age groups. The low number of spawners of age group IV in 1960 was partially caused by heavier exploitation of age group II and III fish in 1958 and 1959. This trend must be stopped if the trout is to be maintained from natural reproduction.

Spawning mortality must be considered with the introduction of a minimum size limit. Ball and Cope (1961) found an average spawning mortality of 48 percent and stated that initial spawners made up most of each spawning run. Spawning mortality is undoubtedly the main source of natural mortality for the catchable-size stock. For efficient exploitation, therefore, it may appear desirable to catch as many fish as possible before spawning. The biological data conflict on this subject. On one hand, the data on sexual maturity suggest that essentially all trout are mature at age IV. On the other hand, fish of age group V exceeded 50 percent of the spawning runs in some streams for several years and rarely amounted to less than 25 percent of the total in any spawning run. Ball and Cope (1961) reported that repeat spawning is four times as common in alternate years as in consecutive years, so we must assume that some trout do not mature until age V, as suggested by Bulkley (1961). The preservation of a moderate number of age group V fish for spawning purposes is necessary, therefore, to maintain suitable spawning stocks. The low spawning runs in Pelican, Arnica, and Chipmunk Creeks in 1960 were caused partially by a lack of age group V fish. The management of this stock according to equilibrium yield will allow escapement of more spawners than necessary when several strong year classes are produced consecutively, but this sacrifice is necessary to allow escapement from weak year classes.

Reduction of creel limit

A reduction of the creel limit from 3 to 2 fish appears to be the simplest way to reduce the catch. A reduction in the creel limit can theoretically reduce the total catch, distribute the catch among more fishermen, or accomplish both. A reduction in total catch is expected under the assumptions that fishermen who consistently catch the creel limit cannot keep as many fish and the low creel limit will not attract as many fishermen from nearby states to Yellowstone. The catch will not be reduced significantly if a reduction in catch by the more skilled fishermen merely provides additional fish for the less skilled anglers or if the fishing pressure increases. A creel limit reduction will not influence all segments of the sport fishery on Yellowstone Lake equally; the probable effects on the shoreline fishery, boat fisheries, Fishing Bridge fishery, and total catch are discussed below.

Shoreline fishery.—In 1953, the creel limit was reduced from 5 to 3 fish, and the fishing season on the lake was opened 2 weeks later. Cope (1957a) examined the shoreline census and reported that more fish were caught under the 3-fish limit in 1953 than under the 5-fish limit in 1952. He concluded that the change in regulations resulted in a redistribution of the shoreline catch so that a larger percentage of the fishermen caught fish than before. It is possible that much of this redistribution was the result of a change in availability of the stock or in angling efficiency. There was an increase in availability from 1952 to 1953 as shown in figure 2. The transition to spinning gear was probably not sufficiently abrupt to cause all of the change in catch distribution. We expect that both the reduced creel limit and the increased availability contributed to the change.

Some effects of reduced creel limits on distribution of the catch among shoreline fishermen can be projected from catch data collected from 1951 to 1960 (table 15). One phase of the shoreline census involves direct observation of fishermen to record their catch and the length of their daily fishing trip. Many fishermen are observed for their entire fishing day, but some move out of the observation area to fish elsewhere. Consequently the data are not total daily catches per fisherman, but catches per fisherman while under observation. This difference does not affect comparisons of catch distribution among years. In 1951 and 1952, one-fourth of the observed fishermen caught one or more fish each. The proportion of successful fishermen (those with one or more fish) increased to one-third in 1953 and was usually about 40 percent in ensuing years. Mean catch per fisherman actually increased with the smaller creel limit, probably owing mostly to increased angler efficiency through the introduction of spinning gear. From 1953 to 1959 there were more fishermen who caught a limit of 3 fish than those who caught only 2 fish. In 1960 this relation was reversed; the mean catch per fisherman dropped to a new low and the percentage of fishermen who caught no fish increased. The pattern established in 1960 reflected the depleted condition of the stock from overfishing. The total catch continued to increase until 1959 as shown in table 3. Thus the reduction in creel limit in 1953 had little influence on the total catch, since the fishing pressure rose from 1953 to 1960. A reduction in the creel limit will not influence the total shoreline catch greatly, unless it reduces the incentive to fish more than it is possible to foresee.

Boat fisheries.—Boat fishermen are more effective than shoreline fishermen as shown in the catch-per-man-hour data in table 4, and in the high catch-per-angler-day data in table 16. The catch per angler-day would be still higher if boat anglers kept all the fish they caught. As a reduced creel limit will not influence all boat fishermen the same, the various segments of the boat fishery are discussed separately.

The rental rowboat fishery is almost entirely limited to inshore areas within a few miles of each boat dock; the boats are small, and there are few outboard motors available for rent. The catch from this fishery dropped when the creel limit was reduced from 5 to 3 in 1953, but there was a gradual increase to 1957 and then a leveling-off until 1959. The total catch has never been restored to the 1952 high of 74,761 fish, in spite of the fact that the catch per angler-day has not changed to a great degree. A reduction in the creel limit will mean more fishing trips per rental boat, but the average trip will take less time. Some reduction in the catch by the rental boat fishery can be expected.

The catch of the guide-boat fishery reached a maximum of 46,550 fish in 1956 and has not been below 32,000 since 1954. This fishery is limited both by the number of available boats and by the fact that few anglers like to pay the higher costs of guide service. We do not expect this

Table 15.—*Percentage distribution of fishermen according to number of fish caught while under observation by census clerks, by years, 1951 to 1960*

Year	Number of fishermen observed	Percent of fishermen who caught—						Mean catch per fishermen
		0 fish	1 fish	2 fish	3 fish	4 fish	5 fish	
1951	533	75.8	10.1	5.6	3.0	1.3	4.3	0.458
1952	819	74.3	11.8	5.5	3.2	1.8	3.3	.561
1953	1,041	63.6	12.8	9.2	14.4			.744
1954	1,146	56.2	18.5	12.1	13.2			.823
1955	1,068	48.4	15.3	15.1	21.2			1.091
1956	880	63.2	16.9	9.7	10.2			.669
1957	722	60.5	17.9	9.0	12.6			.737
1958	565	59.5	14.5	11.0	15.0			.815
1959	1,377	59.7	20.3	9.9	10.1			.704
1960	1,158	71.6	16.1	7.3	5.0			.457

TABLE 16.—*Estimated angler-days, catch, and catch per angler-day for specified segments of the fishery on Yellowstone Lake, by years, 1950 to 1961*

Fishery segment	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961
Shoreline: [1]												
Angler-days	50, 260	114, 743	110, 997	82, 447	71, 088	87, 156	115, 931	86, 060	90, 363	158, 173	123, 786	133, 534
Catch	55, 370	48, 966	53, 423	64, 116	71, 919	97, 831	65, 383	63, 286	81, 210	140, 102	65, 539	98, 954
Catch per angler-day	1. 10	0. 42	0. 48	0. 78	1. 01	1. 12	0. 18	0. 73	0. 90	0. 89	0. 53	0. 74
Rental rowboats:												
Angler-days	37, 488	39, 633	35, 523	28, 216	28. 150	31, 651	28, 081	37, 783	37, 009	36, 449	26, 154	33, 501
Catch	55, 123	60, 993	74, 761	38, 772	39, 969	47, 986	47, 765	61, 350	56, 552	66, 281	41, 266	53, 362
Catch per angler-day	1. 47	1. 54	2. 10	1. 37	1. 42	1. 52	1. 70	1. 62	1. 53	1. 82	1. 58	1. 59
Guide boats:												
Angler-days	6, 523	7, 005	6, 605	9, 022	11, 669	15, 004	18, 107	17, 031	16, 715	14, 733	13, 295	18, 348
Catch	28, 228	29, 449	27, 449	23. 592	32, 400	41, 484	46, 550	43, 436	36, 802	38, 996	34, 854	46, 433
Catch per angler-day	4. 33	4. 20	4. 16	2. 61	2. 78	2. 76	2. 57	2. 55	2. 20	2. 65	2. 62	2. 53
Private trailer boats:												
Angler-days	13, 603	18, 984	25, 255	21, 413	21, 227	33, 237	46, 701	48, 299	61, 002	55, 407	53, 159	49, 363
Catch	50, 377	54, 857	75, 457	50, 118	51, 222	80, 270	110, 937	115, 141	149, 667	124, 421	126, 109	93, 632
Catch per angler-day	3. 70	2. 89	2. 99	2. 34	2. 41	2. 42	2. 38	2. 38	2. 45	2. 25	2. 37	1. 90
Private cruisers: [2]												
Catch	4, 500	5, 052	9, 322	13, 887	13, 351	12, 320	15, 462	13, 806	14, 800	16, 696	18, 513	15, 794
Fishing Bridge: [2]												
Catch	6, 417	8, 938	4, 865	5, 388	7, 072	6, 165	4, 124	4, 136	9, 996	6, 971	8, 617	4. 419
Total catch	200, 015	208, 255	245, 277	195, 873	215, 933	286, 056	290, 221	301, 155	349, 027	393, 467	294, 898	312, 594

[1] Data of shoreline actually represents number of "angler stops" rather than "angler days" as explained in Moore, Cope, and Beckwith (1953). True estimates of angler-days are lower than shown.

[2] Angler-day data not available.

fishery to expand, because many of the anglers who formerly used guide service have purchased their own boats, and a lower creel limit may further reduce the incentive to pay for guide services. As suggested in table 16, most guide-boat anglers had limit catches under either a 5-fish or a 3-fish limit. A reduction in the creel limit will reduce the guide-boat catch significantly.

The greatest increase in catch during the study period was in the trailer-boat fishery (table 16). The catch from this fishery dropped when the creel limit was first reduced to 3 fish, but the catch has continued to rise since 1954 owing to increased angling effort. This is coincident with a national boating trend. Trailer-boat fishermen are effective anglers, and they can reach lightly exploited areas. A reduction in the creel limit will reduce the catch per angler-day, but if the fishing pressure from boats continues to increase, the total catch will not drop. The new boating facilities being constructed at Yellowstone Lake may encourage more boats, but a lower creel limit may discourage many boat fishermen from nearby States. A National Park Service survey revealed that 90 percent of the boats launched on Yellowstone Lake were used primarily for fishing. One influence of a reduction of the creel limit will be to lower the "meat fishing" incentive that has been common among some trailer-boat anglers.

The private-cruiser fishery refers to the large private boats docked at Yellowstone Lake the entire fishing season. The catch from these boats has increased at a moderate rate. A reduced creel limit will undoubtedly decrease the total catch from these boats since limit catches are common. The possible closure of the south arms of Yellowstone Lake to motor-propelled boats may cause a further reduction in the number of boats in this fishery since these boat owners prefer the isolated portions of the lake.

Fishing Bridge fishery.—The Fishing Bridge catch is governed by the number of fish in the spawning runs which pass under the bridge, since fishing pressure is almost always at the maximum. The fishery is controlled by the limited space on the bridge for anglers and not by the creel limit. As soon as one fisherman completes fishing another moves into his place and commences fishing. A reduced creel limit will not reduce this catch.

Total catch.—Analyses by fishery segments reveal that a creel limit reduction will lower the total catch, but it is not the final answer to equilibrium-yield management. If fishing pressure continues to increase, the catch will again exceed equilibrium yield in a few years. The biggest reduction in catch may result from the reduced incentive for fishermen from nearby States to come to Yellowstone for the express purpose of fishing for meat. This type of use has been decreasing in recent years, but we expect that a limit of 2 fish will be still more effective in this regard.

Fishing for fun

Fishing for fun is a management concept which emphasizes the recreational values of angling in contrast to maximum production from a fish stock. In the Great Smoky Mountains National Park fishing-for-fun experiment, only "trophy" fish can be retained, although there is no limit on the number of trout that can be caught and released (Lennon and Parker, 1960). Only single-hook artificial lures may be used. The populations in these fishing-for-fun streams cannot withstand heavy fishing pressure if all captured trout are kept.

The fishing-for-fun concept was introduced at Yellowstone Lake by the National Park Service in 1961 on a voluntary basis. Anglers were encouraged to use barbless hooks and to retain only those cutthroat trout that were badly injured. The creel limit of 3 fish was retained. There is neither a need nor is it desirable to carry the concept to the degree mentioned for the Great Smoky National Park. Yellowstone Lake is excellent trout habitat, and the fish population can withstand a large annual catch. Also, "trophy" fish will never be produced in this lake because of the growth rate and high spawning mortality.

The success of the Yellowstone program depends upon angler cooperation rather than regulation. Data were collected in 1961 to determine angler acceptance of the program and the degree of hooking mortality that could be expected from catching and returning fish to the water. Analysis of interviews with 2,661 shoreline and boat anglers revealed that (1) 82 percent of the anglers interviewed were successful, (2) 80 percent of the successful anglers kept all the fish they caught, (3) 65 percent of the anglers who returned fish still kept their limits, and (4) only 3.9 percent of the successful anglers returned all fish captured. Many anglers have always released trout caught in Yellowstone Lake, and it was not possible to determine how much influence the fishing-for-fun campaign had on the fishing public.

The 1961 interview data indicate that anglers used the program primarily as an excuse to continue fishing after catching their limits. If this interpretation of fishing-for-fun becomes established, the program may induce additional mortality on the stock from hooking, with no reduction in total catch. Preliminary hooking studies conducted during 1961 revealed that mortality from catching and releasing trout with standard spinning gear and treble-hook spoons was negligible (0.7 percent) during the spawning season but reached significant proportions (21.3 percent) in midsummer.

Evaluation of the fishing-for-fun program should continue because it is not possible to predict its effects on management of the fish stock at this time. The program as presently functioning is not an effective tool for reducing the catch.

Effects of regulations

It is not possible to predict what the annual catch would be under any of the regulations mentioned. Variations in effective fishing effort and fishing pressure will require continuous evalution. The knowledge of the equilibrium yield of Yellowstone Lake will never mean that regulations to attain that yield can be established a year in advance of an angling season unless some quota system is possible to administer. Its greatest value is that it sets a goal on the number of fish that can be removed and allows the fishery manager to distribute the catch or fishing pressure as considered expedient.

The principal aim of fishery management on Yellowstone Lake should be to provide the maximum number of fishing hours or trips and not to derive the maximum possible yield in pounds or numbers of fish. There is the problem in arriving at a happy medium between attaining maximum use of the resource and avoiding extended overexploitation of it. A few anglers do not care about keeping any fish, but the majority wish to retain a few trout for eating. Management must be directed at the majority if we are to provide the maximum number of fishing trips. The question is what regulations are necessary to prevent overfishing on Yellowstone Lake without reducing the fishing incentive for the majority of anglers. For the present, a 2-fish creel limit and a 12-inch minimum size limit should essentially accomplish both aims. Only continuous evaluation of the fishing pressure and the condition of the stock will answer this question fully.

Redistribution of fishing pressure

The present calculated equilibrium yield is below the potential equilibrium yield for Yellowstone Lake because of the poor distribution of fishing pressure. If Yellowstone Lake were to be managed for fishing purposes only, additional access to the southern areas of the lake would be necessary. The National Park Service is considering a plan to close the two southern arms of the lake to all motor-propelled boats to perpetuate wilderness values. A partial closure was carried out in 1961. If these arms are closed, fishing pressure in the southern area will likely be lower in the future than at present.

The effects of this closure cannot be accurately predicted. It may shift additional fishing pressure to the northern area and West Thumb which would require a lower equilibrium yield for the lake than the present estimate of 325,000 fish. Present methods of measuring changes in the stock are adequate to determine the degree of exploitation in these two areas, and continuous measurement of the stock condition is imperative.

Interspecific competition

The longnose sucker and redside shiner must be considered possible detriments to cutthroat trout production in Yellowstone Lake. Several workers (Bassett, 1957;[5] Rawson and Elsey, 1948; Larkin and Smith, 1953) found that these species adversely influenced trout populations. Competition may be expected to increase as the density of the trout population is reduced. The only long-term sampling of suckers of Yellowstone Lake has been in fish traps where every sucker was killed. Trap counts have decreased in recent years, but this decrease is not necessarily representative of the lake sucker population. Biesinger[6] studied the distribution and degree of competition between trout and introduced species in Yellowstone Lake. The sucker is distributed widely and presently occupies all the suitable habitat. The best sucker habitat is in the highly productive littoral zone which is heavily used by trout, and suckers feed on essentially the same food organisms as trout. The influence of suckers on the cutthroat trout stock is not clear. Available data are insufficient for determining whether the decrease in trout density in heavily used fishing areas has resulted in an increase in suckers, although growth rates of suckers did not increase with a decrease in trout density.

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[5] "Further life history studies of two species of suckers in Shadow Mountain Reservior, Grand County, Colorado," by Howard M. Bassett. Unpublished master's thesis, Colorado State University, 1957, 112 pp.

[6] "Studies on the relationships of the redside shiner (*Richardsonius balteatus*) and the longnose sucker (*Catostomus catostomus*) to the cutthroat trout (*Salmo clarki*) population in Yellowstone Lake," by Kenneth Biesinger. Unpublished master's thesis, Utah State University, 1961. 90 pp.

The redside shiner distribution is more limited than that of suckers, possibly because the shiners were introduced into Yellowstone Lake more recently. Their principal habitat is the shallow lagoons and a few tributary streams in the northern area. They share the habitat with small cutthroat trout, and their feeding habits are similar to those of small trout. No redside shiners have been collected in the limnetic zone of the lake, and none were found in trout stomachs. Biesinger [7] concluded that competition is more severe between juvenile cutthroat trout and longnose suckers or redside shiners than between the adults. Lake Chubs are confined to a few lagoons and streams and are so rare that competition must be insignificant. The native longnose dace is confined to a few streams, and no competition is evident.

Yellowstone Lake is deeper and has less littoral habitat than many of the other lakes where severe competition between suckers and trout has been found. It is possible, however, that both the longnose suckers and redside shiners will expand their numbers if the cutthroat trout stock is further reduced. Partial control methods such as spot-poisoning or trapping could be tried, but there is reason to believe that a well-managed stock of cutthroat trout is the best answer.

Planting

Yellowstone Lake can produce excellent angling with natural reproduction, and can be managed on this basis. Planting would simplify management in some cases and possibly would increase the yield. Large numbers of eggs, fry, and advanced fry were stocked in Yellowstone Lake and tributaries before 1951 (table 1). All plantings consisted of eggs or fry from Yellowstone Lake spawners. The survival rate of these plantings was not compared with the survival rate of wild fish, but there is no doubt that the plantings were beneficial in several tributary streams. In Pelican Creek, no escapement was reported from 1936 to 1944,[8] and year classes produced during that period must have resulted from plantings; the strong and unnaturally stable spawning runs from 1945 to 1948 enumerated in table 10 must have been the result of plantings. Several substantial spawning runs were established from plantings in small streams, such as Hatchery Creek. The Hatchery Creek runs have disappeared without stocking. There is also no evidence that the egg-collecting operation carried out up to 1953 was influencing the lake stock, although the continuous planting program and the low fishing pressure during the years of heavy egg-collecting prevent accurate evaluation.

From 1945 to 1950 a good escapement was allowed in all sampled streams, but small plantings were made in some streams to supplement natural reproduction. All planting of Yellowstone Lake or tributary streams was terminated after 1950, except for two small experiments in Arnica Creek in 1954 and 1955. The year classes from 1946 to 1950 were the result of plantings plus natural reproduction, and all year classes after 1950 were entirely the result of natural reproduction. From the variations in strength of year classes shown in figure 6, it is doubtful whether the small supplemental plantings from 1945 to 1950 benefited the stock. The plantings in Arnica Creek in 1954 and 1955 did not appear to influence year-class strength in this stream. Note, however, that the spawning runs in table 11, apparently derived entirely from plantings (1945 to 1948), fluctuated less than spawning runs derived from natural reproduction. There is evidence, therefore, that the use of fry plantings in streams to supplement artificial reproduction when environmental conditions in spawning streams are not suitable would be an excellent management tool to offset the great fluctuations in year-class strength that presently occur. Any fry plantings contemplated should be made in early August or after the high water period.

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[7] See footnote 6.

[8] Letter from W. A. Dunn, Superintendent, Yellowstone Hatchery, to Regional Director, U.S. Fish and Wildlife Service, dated March 8, 1945.

Although fry stocking may alleviate the occurrences of poor year classes in some streams, a fingerling stocking program would have greater benefit to the entire lake stock. Most planting experiments (Curtis, 1951) have found a higher survival rate for fingerlings than fry, and we would expect similar results in Yellowstone Lake. Survival rate of wild fish in the fingerling size range in Yellowstone Lake is known to be high. The use of hatchery-reared fingerlings would simplify management, since natural reproduction could be partially desregarded and overexploitation of prespawners would not be as serious.

Fingerling plants in management would be costly, and the lake can be managed rather easily with an average equilibrium yield of about 325,000 trout without stocking. The lake production for the angler could be increased considerably by fingerling stocking, but the esthetic value of fishing a "natural" trout population would be partially lost. A stream fry-planting program can be carried out at low cost, since the fry would have to be fed only a few weeks, and such a program would not reduce the natural aspects of angling as much as fingerling stocking. In any case, only eggs of cutthroat trout from Yellowstone Lake should be used in any stocking plan, to preserve one of the few native stocks of this species in the United States.

YELLOWSTONE RIVER FISHERY

The 15-mile section of the Yellowstone River from a point 300 yards below Fishing Bridge to the Upper Falls furnishes much excellent angling, and the management of this fishery is closely related to management of the lake stock. Regulations should be similar for Yellowstone Lake and this section of the river.

The total catch from the river rose from 19,729 in 1950 to a maximum of 81,724 in 1958, and has fluctuated to a greater degree than that of the lake fishery (table 17). The catch per man-hour was lower from 1951 to 1953 than in recent years, and the higher catch per man-hour after 1953 has been attributed partially to the greater use of spinning gear. The poor catch in 1956 was due to high water (table 17). The best fishing success was recorded in 1955 and was associated with low water. The biweekly catch per man-hour is always greatest at the opening of the season and decreases rapidly toward the end of the season. This trend takes place because the river fishery is almost wholly dependent upon a spawning migration.

TABLE 17.—*Estimated catch and catch per man-hour for Yellowstone River fishery, by years, 1951 to 1959*

[With mean monthly discharge of river, in cubic feet per second, for June and July]

Year	Catch	Catch per man-hour	Discharge	
			June	July
1951	19, 729	0. 236	4, 204	4, 825
1952	20, 603	. 225	4, 796	3, 788
1953	27, 932	. 346	2, 617	3, 798
1954	35, 912	. 478	3, 916	5, 040
1955	63, 701	. 649	2, 851	3, 455
1956	21, 907	. 325	6, 640	5, 172
1957	47, 762	. 446	4, 031	4, 605
1958	81, 724	. 500	3, 361	2, 460
1959	67, 799	. 427	3, 285	4, 387

Some data on the relations of the river stock to the lake stock were determined from tagging fish in the Cascade area (5 miles downstream from Fishing Bridge) of the Yellowstone River in 1951, 1952, and 1953 (Ball and Cope, 1961). Most tagged fish were recovered in the river or in the

Fishing Bridge area when they were captured the same year as they were tagged. A relatively large number captured the year after tagging were caught in the lake. Few fish tagged in Pelican Creek or near the Fishing Bridge campground were captured in the river below Fishing Bridge. This tagging study shows that many of the lake fish move down into the river below Fishing Bridge to spawn and then return to the lake. It is these fish which probably constitute most of the river fishery now. In addition, the river probably contains a small resident population.

From available data, we believe that sound management of the lake stock will automatically manage the river stock if similar regulations are in effect in the two areas. Overexploitation of the resident river stock will be compensated by immature fish produced from the spawning areas around and downstream from Fishing Bridge. The July 1 opening of the fishing season protects the spawning stock moving into this river area for a sufficient part of the spawning season in most years. A reduction of the creel limit to two fish for the river will help to insure adequate seeding of this important spawning area. The minimum size limit of 12 inches recommended for the lake and river would also be of considerable value in protecting the river stock, because the river catch includes more small fish in the latter part of the season than the lake catch.

SUMMARY

This paper summarizes data collected from 1945 to 1961 on the biology of the cutthroat trout in Yellowstone Lake, fluctuations in the fish populations, catch, and management. The principal purpose is to establish a maximum equilibrium yield and to consider management with respect to equilibrium yield.

Biological investigation and fish culture are briefly reviewed. The life history of cutthroat trout in Yellowstone Lake and the ecology of the lake are summarized. The fish stocks from the northern area and West Thumb are considered separately.

The catch increased from 200,015 cutthroat trout in 1950 to 393,497 in 1959. Fishing pressure was more erratic than the catch and reached 526,728 man-hours in 1959. Catch per man-hour did not vary according to stock density and was not a reliable index of stock condition. Spawning concentrations, water levels, and fishing methods influenced catch per man-hour. Most of the trout caught from 1950 to 1961 fell within a 100-mm. range, and mean total lengths ranged from 336.7 to 357.8 mm. Age groups III, IV, and V comprised most of the catch. The northern area included more older fish during the early part of the season than West Thumb; the two areas were similar after the middle of July. Estimated percentages of sexually mature fish in the catch ranged from 64.3 to 84.8.

Growth rate increased from 1950 to 1956, but leveled off from 1956 to 1959. Optimum growth for the catchable stock was reached in 1956.

Spawning runs in six streams were measured from 1945 to 1961. General characteristics of spawning runs are described. Changes in the number and mean age of spawners are related to the increased catch. Arnica Creek runs fluctuated irregularly, but the low runs in 1959, 1960 and 1961 were attributed to overfishing in West Thumb. Pelican Creek has the largest spawning run of the streams measured, and its spawners are subjected to the greatest fishing pressure. The 1960 spawning run was depleted by the increased catch in the northern area. The 1961 run indicated that some recovery

of the fishery occurred due to the reduced catch in 1960.

Clear and Cub Creek data are not complete, but a downward trend in number of spawners has been related to the increased catch. Chipmunk and Grouse Creeks are isolated, and the spawners from these streams are subjected to the least fishing pressure of the sampled streams. These latter streams have a larger number of fish of age group V or older than other streams, and since many age group V are probably initial spawners, the spawning runs can be depleted by fishing more easily. The low 1960 and 1961 runs are definitely attributed to overfishing.

Accurate mortality data could not be computed, but total instantaneous mortality rates are calculated by year class for fish of age groups IV and V to show gross changes. Excessive mortality occurred in the northern area when the catch exceeded 200,000 trout, and in West Thumb when the catch exceeded 100,000.

Year classes in the northern area were strongest in 1945, 1949, 1953, and 1957. The West Thumb catch was more irregular than the northern area catch, but strong year classes were produced in 1947, 1952, 1953, 1955, and 1957.

Methods for determining maximum equilibrium yield are described. Three stages of exploitation are defined for the Yellowstone stock; these stages consider growth rate, spawning runs, and age groups in the catch. Overfishing commences in the second stage of exploitation. The estimated maximum equilibrium yield averaged 325,000 fish and may range from 290,000 to 340,000 fish with the present distribution of fishing pressure.

Various measures are discussed for equilibrium yield management. Control of fishing pressure is the most precise but is not practical. Delaying the fishing season until July 1 would be quite unpopular, even though it would assure survival of more fish through spawning. A minimum size limit of 12 inches is recommended to reduce and redistribute the total catch, and to protect prespawning fish. A reduction in creel limit will reduce the total catch among some fishermen, but its greatest value may be to reduce the incentive to fish for food. The fishing-for-fun management system is discussed. Angler interview data suggest that a voluntary system is not a positive measure for reducing the catch. Hooking experiments showed that additional mortality will be inflicted on the fish population by catching and releasing fish after the spawning season. The effects of all regulations should be observed closely.

Closure of the south arms of Yellowstone Lake to motor-propelled boats may result in a lower equilibrium yield for the lake.

Competition from the longnose suckers and redside shiners may increase, but good trout management and the small amount of sucker and redside shiner habitat can prevent a population explosion of these introduced species.

The planting of cutthroat trout fry to prevent the occurrence of poor year classes is considered a possible management tool. Fingerling stocking would simplify management but is not recommended at this time.

The Yellowstone River fishery is reviewed. It is closely connected with the lake population, and wise managment of the lake should automatically manage the river population. Similar regulations should be in effect on both areas.

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Appendix A—DISTRIBUTION OF FISHING

Creel census information has been collected by segments, and the methods for determining the percentage of fish from each segment that came from the northern area or from West Thumb is described below: The methods for conducting the census were described by Moore et al. (1952).

Rented rowboats

The rented rowboats from Fishing Bridge and Lake Dock are used entirely in the northern area and the entire catch from these docks is known to come from the northern area. The rented boats from West Thumb dock fish entirely in the West Thumb area.

Fishing Bridge

The entire catch is from the northern area.

Guide boats

Fishing Bridge guides from 1950 to 1953 fished in the southern area as follows: 1950, 4.5 percent; 1951, 12.1 percent; 1952, 1.2 percent; 1953, 4.0 percent. The remaining catch came from the northern area.

Lake guides fish principally in the northern area, but a census of guide boats showed that 3 percent of the catch came from the southern area from 1954 to 1960. The remainder, or 97 percent, was from the northern area.

West Thumb guides fish mostly in West Thumb, although an average of 20 percent, as determined from guide boat records, fish in the southern area that adjoins West Thumb. This percentage varies slightly annually, but accurate records are not available for all years, and we have assumed that 80 percent of the catch comes from West Thumb and 20 percent from the southern area.

Private boats

The trailer boat fishery is the most difficult to break down accurately into areas. Boat card data have been used to determine where boats were launched and the total catch per launching area. All boat launching areas are in the northern area and West Thumb. The increase in average horsepower per boat in recent years has enabled boats to reach more inaccessible areas than was possible in previous years. Consequently there has been an increase in fishing pressure in the southern area. The percentages in table A–1 give the estimated distribution of the catch by areas from 1950 to 1961 and take into account all these known changes.

The cruiser fishery includes the largest boats on the lake, and all are anchored the entire fishing season at Lake, Fishing Bridge, and West Thumb docks. These boats fish mostly in the southern area. The estimated distribution of the cruiser catch by areas is: northern, 15 percent; West Thumb, 10 percent; and southern, 75 percent.

Lake shoreline

Accurate data are available on the shoreline fishery since fisherman counts are made three times a week along the entire accessible shoreline. The shoreline catch was divided between the northern area and West Thumb from 1950 to 1961 as shown in table A–2.

Appendix table A–1.—*Estimated percentage distribution of trailer boat catches, by area, 1950 to 1961*

Year	Percent of catch from—		
	Northern area	Southern area	West Thumb area
1950	80.0	2.0	18.0
1951	79.0	1.0	20.0
1952	65.5	3.8	30.7
1953	65.0	4.2	30.8
1954	64.2	4.8	31.0
1955	64.2	4.8	31.0
1956	63.5	5.5	31.0
1957	62.2	6.8	31.0
1958	62.8	7.2	30.0
1959	61.3	8.2	30.5
1960	61.0	8.3	30.7
1961	61.0	8.3	30.7

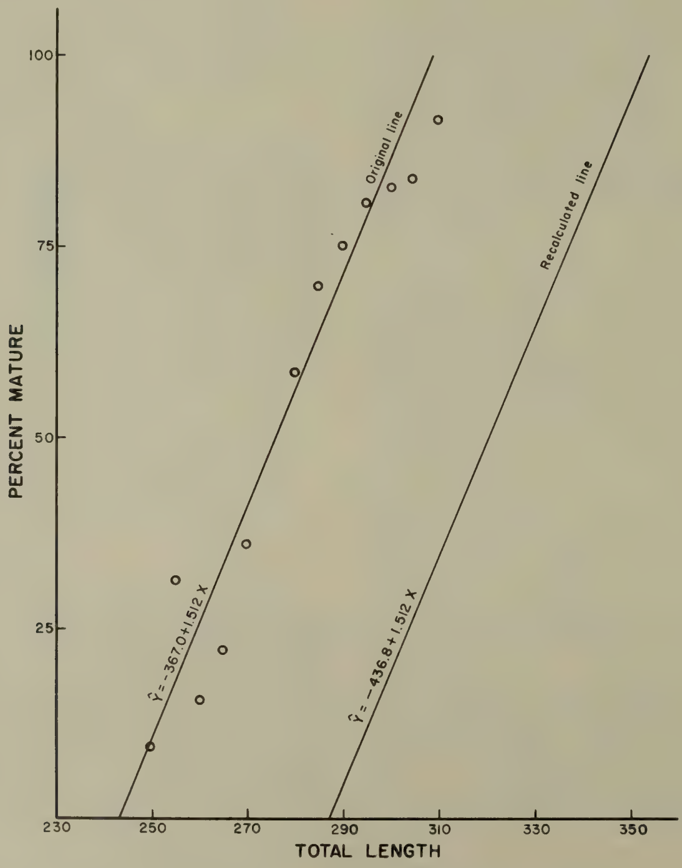
Appendix table A–2.—*Percentage distribution of shoreline catches, by area, 1950 to 1961*

Year	Percent of catch from—	
	Northern area	West Thumb area
1950	72.7	27.3
1951	71.5	28.5
1952	77.7	22.3
1953	75.3	24.7
1954	75.1	24.9
1955	76.6	23.4
1956	74.6	25.4
1957	75.9	24.1
1958	71.9	28.1
1959	76.4	23.6
1960	80.5	19.5
1961	74.1	25.9

Appendix B—MATURITY OF FISH IN CATCH

A sample of 1,055 fish from the catch at Fishing Bridge dock in 1953, 1954, and 1960 and at West Thumb dock in 1960 was examined to determine the maturity of fish in different length groups up to July 15. A mature fish is one which has spawned in previous years or has spawned or will spawn in the season of capture. The differences between sexes were so minor that they have been combined. From these data, no fish below 250 mm. was mature and no fish above 305 mm. was immature. Data for each year gave the same results. A regression line was calculated from combined data to determine the expected percentage of mature fish in each 5-mm. length group in the 245 to 310 mm. range (fig. B–1).

Stage of maturity was difficult to measure after July 15, but growth is rapid during the angling season and this regression line could not be applied accurately to the entire season's catch. Many trout captured in August measuring 325 mm. total length were known to be immature. Bulkley (1961) studied growth rates of cutthroat trout, but he was not able to measure seasonal periodicity of growth. To compensate for growth we assumed that half the season's growth will be completed by August 1. One-half of the mean annual growth



Appendix figure B–1.—Relation of maturity to total body length in millimeters. Original line from catch data collected before July 15 in 1953, 1954, and 1960. Recalculated line includes adjustment for expected seasonal growth.

increments between age groups II and III and age groups III and IV, or 46 mm., has been considered the expected growth rate up to August 1. The regression line calculated earlier was moved 46 mm. to the right. Actually most of the catch is made during June and July, and the use of this regression line may indicate a larger percentage of immature fish than is true. From our knowledge of the sizes and ages of fish entering the spawning runs, the second regression line represents the average maturities of the catch accurately. The percentage of mature fish in each 5-mm. length group was taken from the recalculated regression line and applied to the length distribution of the catch each year (table B–1). The sum of the mature fish in all length groups was then divided by the total sample to obtain the percent of mature fish in the catch.

Appendix table B–1.—*Percent of mature fish by 5-millimeter length groups as taken from regression line on figure B–1*

Length group	Percent mature
285 mm	0.0
290 mm	4.3
295 mm	11.9
300 mm	19.6
305 mm	27.2
310 mm	34.8
315 mm	42.4
320 mm	50.0
325 mm	57.6
330 mm	65.2
335 mm	72.8
340 mm	80.4
345 mm	88.0
350 mm	95.6
355 mm	100.0

Appendix C—GROWTH INCREMENTS

APPENDIX TABLE C–1.—*Increments of growth of age groups II to VI from Fishing Bridge, and deviations from mean growth, by calendar year, 1946 to 1960*

[In millimeters; number of fish in parentheses]

Age group and year of life	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	Mean
Age group II:																
Year 2				88		101	80	159	150	104	140		113	139		119.30
Year 1			46		56	47	49	49	50	47		52	45			49.00
			(3)		(14)	(21)	(17)	(9)	(2)	(5)		(15)	(15)			
Age group III:																
Year 3		70		91	120	81	96	89	95	86	94	86	98	98	103	92.84
Year 2	117		86	48	96	108	99	91	117	87	90	81	121	110		96.23
Year 1		47	44	47	44	44	44	49	44	44	44	47	47			45.42
	(11)	(18)	(48)	(145)	(76)	(43)	(60)	(44)	(44)	(29)	(24)	(60)	(46)			
Age group IV:																
Year 4		73		69	80	88	69	85	86	87	94	96	82	105	109	86.38
Year 3	83		87	97	92	112	101	109	87	99	96	100	86	88		95.15
Year 2		60	47	72	76	60	57	83	76	74	54	92	93			70.33
Year 1	45	43	45	45	45	46	44	44	45	44	46	45				44.75
	(110)	(121)	(184)	(110)	(69)	(80)	(103)	(191)	(136)	(39)	(52)	(70)				
Age group V:																
Year 5		93		74	82	75	80	75	79	77	76	68	81	68	71	76.85
Year 4	45		84	68	78	74	93	77	78	84	99	104	90	94		82.15
Year 3		92	102	94	95	80	94	91	99	92	106	82	96			93.58
Year 2	54	37	40	50	56	57	58	50	55	51	94	79				56.75
Year 1	43	45	44	44	45	44	43	44	46	47	43					44.64
	(129)	(110)	(126)	(90)	(123)	(80)	(110)	(113)	(20)	(23)	(23)					
Age group VI:																
Year 6				54	52	57	44		52		65	46		51		52.63
Year 5			82	79	80	88		86		97	93		94			87.37
Year 4		65	75	68	77		73		92	77		75				75.26
Year 3	94	84	83	96		93		112	93		135					98.75
Year 2	36	48	54		38		22	45								40.50
Year 1	43	43		44		43	44									43.40
	(16)	(22)		(26)		(7)	(12)		(2)							
Percentage deviation from mean, all ages	−4.06	−10.73	−3.09	−5.69	+1.21	−2.04	−2.13	+0.43	+1.93	+0.72	+6.30	+4.46	+9.24	+6.53	+15.54	

APPENDIX TABLE C-2.—*Increments of growth of age groups II to VI from West Thumb, and deviations from mean growth, by calendar year, 1946 to 1960*

[In millimeters; number of fish in parentheses]

Age group and year of life	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	Mean
Age group II:																
2				122		113	127	128	107				140	144	116	124.62
1			50		50	56	50	49				48	50	56		51.10
			(27)		(14)	(7)	(28)	(25)				(4)	(18)	(4)		
Age group III:																
3				86	101	104	82	93	93	97	87	85	85	93	92	91.50
2			89	88	97	97	83	90	108	97	104	102	100	117		97.65
1		45	49	51	49	48	46	53	44	44	46	47	48			47.50
		(145)	(124)	(166)	(107)	(130)	(118)	(89)	(39)	(23)	(28)	(62)	(47)			
Age group IV:																
4		85		87	84	82	90	88	96	89	91	104	106	105	111	93.69
3	113		99	98	96	103	101	95	82	99	104	81	95	86		96.30
2		61	63	65	66	59	63	85	75	65	89	81	95			72.25
1	46	45	47	46	45	44	49	43	44	44	47	46				45.50
	(105)	(149)	(189)	(155)	(200)	(145)	(135)	(137)	(120)	(56)	(61)	(70)				
Age group V:																
5		85		70	76	76	80	85	81	72	85	83	82	69	75	78.38
4	70		87	82	75	72	73	76	82	84	90	98	91	99		83.00
3		98	91	86	90	103	94	90	96	99	114	115	92			97.32
2	48	49	51	45	27	45	60	47	52	53	69	69				51.25
1	44	44	43	43	43	46	43	44	45	44	48					44.27
	(78)	(111)	(70)	(88)	(70)	(65)	(105)	(85)	(16)	(17)	(24)					
Age group VI:																
6				51	55	52	44	50	62		52	55		56		53.00
5			98	95	88	95	90	78		70	102		117			92.55
4		62	72	75	70	70	61		97	55		81				71.44
3	88	84	115	79	95	84		98	88		72					89.22
2	26	26	45	22	36		47	56		36						36.75
1	47	44	43	43		43	44		43							43.85
	(18)	(18)	(9)	(9)		(6)	(4)		(4)							
Percentage deviation from mean, all ages	−0.80	−5.64	−0.20	−5.65	−6.52	−4.80	−4.09	+0.38	+0.27	−2.28	+6.27	+5.43	+7.57	+6.11	+8.19	

Appendix D—YEAR-CLASS STRENGTH

Appendix table D–1.—*Calculation of year-class strength from method of el-Zarka (1959) for northern area and West Thumb*

Area and year class	Age groups included	Sum, first year	Sum, second year	Mean	Difference	Percentage difference between years	Relative strength, first year	Final rank, first year
Norther area:								
1944–1945	VI-VII	10.7	13.5	0.7	2.8	23.1	0.0	−0.2
1945–1946	V-VI	50.7	39.7	12.1	−11.0	−24.3	23.1	22.9
1946–1947	IV-VI	77.6	63.5	45.2	−14.1	−20.0	−1.2	−1.4
1947–1948	III-VI	76.6	92.4	70.6	15.8	18.7	−21.2	−21.4
1948–1949	II-VI	93.4	111.7	84.5	18.3	17.8	−2.5	−2.7
1949–1950	II-VI	111.7	99.8	102.6	−11.9	−11.2	15.3	15.1
1950–1951	II-VI	99.8	87.0	105.8	−12.8	−13.7	4.1	3.9
1951–1952	II-VI	87.0	105.3	93.4	18.3	19.0	−9.6	−9.8
1952–1953	II-VI	105.3	113.0	96.2	7.7	7.1	9.4	9.2
1953–1954	II-VI	113.1	77.2	95.5	−35.9	−37.6	16.5	16.3
1954–1955	II-V	76.8	80.9	78.8	4.1	5.2	−21.4	−21.6
1955–1956	II-V	80.9	82.0	81.5	1.1	1.4	−16.2	−16.4
1956–1957	II-IV	70.5	101.3	85.9	30.8	35.9	−14.8	−15.0
1957							21.1	20.9
West Thumb:								
1944–1945	VI	4.0	2.2	3.1	−1.8	−58.1	0.0	58.3
1945–1946	V-VI	32.6	24.9	28.8	−7.7	−26.7	−58.1	0.7
1946–1947	IV-VI	49.7	67.5	58.6	17.8	30.4	−84.8	−26.0
1947–1948	III-VI	101.7	93.0	97.4	−8.7	−8.7	−54.4	4.4
1948–1949	II-VI	99.4	99.3	99.4	−0.1	−0.1	−63.1	−4.3
1949–1950	II-V	96.8	95.9	96.4	−0.9	−0.9	−63.2	−4.4
1950–1951	II-V	95.9	92.8	94.4	−3.1	−3.3	−64.1	−5.3
1951–1952	II-VI	94.9	122.4	108.7	27.5	25.3	−67.4	−8.6
1952–1953	II-VI	120.8	119.3	120.0	−1.5	−1.3	−42.1	16.7
1953–1954	II-VI	119.3	73.8	96.6	−45.5	−47.1	−43.4	15.4
1954–1955	III-VI	73.8	93.2	83.5	19.4	23.2	−90.5	−31.7
1955–1956	II-V	93.1	89.8	91.5	−3.3	−3.6	−67.3	−8.5
1956–1957	II-IV	75.9	90.2	83.1	14.3	17.2	−70.9	−12.1
1957							−53.7	5.1

Appendix E—CATCH CURVES

Appendix table E–1.—*Estimated number of fish caught from northern area and from West Thumb, by age group, 1950 to 1961*

Calendar year	Age group II		Age group III		Age group IV		Age group V		Age group VI		Age group VII		All age groups	
	Northern area	West Thumb	Northern area	West Thumb	Northern area	West Thumb	Northern area	West Thumb	Northern area	West Thumb	Northern area	West Thumb	Northern area	West Thumb
1950	1,448	3,109	18,971	16,613	54,885	12,047	53,872	14,767	14,626	1,943	1,014	97	144,816	48,575
1951	2,960	833	19,980	17,657	50,320	21,199	53,724	11,094	19,980	1,146	1,036	156	147,999	52,085
1952	5,108	1,789	52,609	20,954	66,910	23,893	39,840	14,055	5,789	2,300		894	170,255	63,885
1953	7,642	776	27,590	11,646	39,895	16,886	45,724	7,647	7,901	1,863	777		129,529	38,819
1954	10,965	3,330	27,856	15,305	44,599	23,628	58,231	10,364	6,520	1,074			148,171	53,702
1955	5,749	5,323	38,521	25,049	51,553	30,841	79,150	14,872	16,673	1,957		235	191,647	78,276
1956	14,643		33,672	26,374	78,690	39,950	61,488	19,283	7,686	865			182,999	86,472
1957	2,624	645	23,058	12,443	100,292	43,690	57,738	33,459	3,749	1,936			187,462	92,174
1958	2,190	2,103	22,999	13,985	101,414	51,000	83,453	36,384	8,761	1,682			219,036	105,155
1959	20,275	1,523	50,062	23,669	136,169	79,676	43,554	12,186	250	117			250,310	117,171
1960	5,806	3,523	58,775	25,528	89,796	46,157	26,486	9,541	725	1,204			181,588	85,953
1961	1,730	1,651	51,513	26,318	116,864	57,491	22,104	11,557		97			192,211	97,114

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SEA-WATER SYSTEMS FOR EXPERIMENTAL AQUARIUMS

A Collection of Papers

Edited by
John R. Clark and
Roberta L. Clark

RESEARCH REPORT 63

UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, *Secretary*
Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*
FISH AND WILDLIFE SERVICE
Clarence F. Pautzke, *Commissioner*
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Daniel H. Janzen, *Director*

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NOTE

The Bureau of Sport Fisheries and Wildlife does not endorse particular products or equipment. References to trade names and manufacturers of equipment in the accounts by various laboratories of their experiences in setting up salt-water aquariums have been retained for identification. The Bureau neither recommends nor disapproves the products referred to.

SEA-WATER SYSTEMS FOR EXPERIMENTAL AQUARIUMS

This volume was created to provide a source of information on design, construction, and operation of experimental aquarium facilities. The idea developed out of our own frustrated search for published material on sea-water supply systems when we were planning facilities for the new Sandy Hook Marine Laboratory. We found that we had to spend many weeks visiting laboratories and many months corresponding with aquarists to obtain the information we needed. It became clear, as a result of this, that nearly everyone who has built a sea-water system has experienced similar difficulties.

The solution to this problem suggested by J. L. McHugh of the U.S. Bureau of Commercial Fisheries was that we prepare a volume on sea-water systems from our visits and correspondence. We decided rather to sponsor a compilation of papers contributed by the aquarists themselves. In soliciting papers, we asked contributors to describe their experiences in solving problems encountered in designing, constructing, and maintaining systems, rather than give detailed and comprehensive accounts of complete sea-water systems. Our letter of invitation to potential contributors stated:

> We visualize such a publication as addressed primarily to the biologist and secondarily to the engineer and containing accounts of personal experiences illustrated by simple sketches of general layout or special features. Annotated descriptions of recirculating systems, holding tanks, rearing ponds, or other facilities would be pertinent. Of great value would be descriptions of methods for combating such problems as fouling, siltation, equipment failure, and toxicity of system components. Discussions of such factors as selection of pumps and advantages of continuous versus interrupted supply would be appropriate also. Discussions of past or present shortcomings of systems and possible remedies would also be helpful to others planning experimental facilities. Contributions would not have to be lengthy to be of value. I believe the readers of such a volume would be interested in simple systems as well as those of more elaborate design.

We attempted to embrace as great a variety of experience in sea-water aquarium problems as possible. That some success was achieved in this aim is evident from the scope of the contributed papers. Geographic coverage extends from the tropics to temperate latitudes and from the mid-Pacific Ocean to the eastern Atlantic Ocean. Environmental coverage extends from estuarine to open coastal emplacements. System coverage extends from small recirculating tanks to continuous-supply systems of hundreds of gallons per minute. Nearly every problem which could arise with sea-water systems is explored in one paper or another.

In attempting broad scope, we have included papers ranging from the philosophical to the very practical. Thus, all concerned, from experimental biologists to maintenance personnel, should find this volume useful. The comprehensive index was included to enhance its value as a reference work and as a manual for design, construction, operation, and maintenance of all types of experimental sea-water supply systems.

John R. Clark, *Marine Biologist.*
Roberta L. Clark, *Editorial Assistant.*

SOME PRINCIPLES AND PRACTICES OF WATER MANAGEMENT FOR MARINE AQUARIUMS

By James W. Atz, *Curator*

New York Aquarium, New York Zoological Society, Brooklyn, N.Y.

Abstract.—Instability of sea water and its organic constituents, when confined in aquariums or circulatory systems, and the characteristic inability of marine organisms to adjust to changes in their environment, combine to make the keeping of marine life in captivity a difficult procedure. The maintenance of sea water in suitable condition depends upon a chemically inert water system, a low ratio of animal life to volume of water, the control of bacteria, and the elimination of metabolic waste products. Methods used to accomplish this include aeration, filtration, storage in the dark, and treatment with alkalizers, ultraviolet light, and antibiotics.

Despite a tradition that goes back to the ancient Romans and a lively, uninterrupted history of more than a century, the keeping of marine animals in captivity remains more of an art than a science. The most valuable attribute an experimentally minded marine biologist can have is a wet thumb. Of course, the more he is able to apply precise, reproducible techniques to the maintenance of his subjects, the greater are his chances of success, but there will inevitably remain some intangibles, indescribable and unpredictable, that he, as scientist, can only attribute to the complexity of the subject he is dealing with.

Central to the problem of keeping marine animals alive in aquariums is the maintenance of sea water in such confined bodies. In his classic elucidation of the coordinate roles played by the fitness of the environment and the fitness of the individual in the evolution of life, Henderson (1913) points out that in no other habitable place on the earth are so many conditions so stable and so enduring as in the ocean. This eon-embracing stability of the sea as an environment of life is the fundamental cause of the difficulties that marine laboratories, public aquariums, and home aquarists alike have in keeping marine fishes and invertebrates. Because these animals evolved in a world that has remained practically constant in temperature, osmotic pressure, alkalinity, and chemical composition, they have developed few mechanisms to isolate themselves from these environmental factors and little tolerance for change. In captivity, they perforce must be provided with an environment that at least approaches in stability their natural one. But once sea water has been removed from the ocean, it begins to change and to lose some of its capacity to support delicate marine life. This is directly and indirectly the result of its no longer being part of a body of water that is to all intents and purposes infinitely large. The best that any marine aquarium practices seem to be able to do is merely to slow down the rate of this deterioration.

The author's present address is American Museum of Natural History, New York, N.Y.

MATERIALS SUITABLE FOR SEA-WATER SYSTEMS

Any discussion of water as an environment, artificial as well as natural, ought at least to mention the attributes that intimately connect this unique substance with life, namely its great specific heat, latent heats of fusion and evaporation, and thermal conductivity, its high surface tension and dielectric constant, its transparency, and its tremendous solvent power combined with chemical stability. These characteristics were first penetratingly analyzed by Henderson (1913), and they have been reviewed by Sverdrup, Johnson, and Fleming (1942). Of them, only water's close approach to being the universal solvent presents any special problems in aquarium management.

Because of water's ability to dissolve a wide variety of toxic substances and because of the extreme sensitivity of most aquatic animals to them, the only certain way to keep water suitable for most marine life is to use chemically inert materials throughout the water system. Copper is a good example of a problem material. This heavy metal is acutely toxic to fresh-water fish in concentrations of a few parts per hundred million, but in hard alkaline waters its toxicity is considerably reduced (Doudoroff and Katz, 1953). In sea water, as might therefore be expected, copper is somewhat less toxic, and at the New York Aquarium and the John G. Shedd Aquarium, prophylactic doses of 0.20 to 0.25 p.p.m. (parts per million) are considered safe for the great majority of marine fishes (Carleton Ray, personal communication; Braker, 1961). At the Steinhart Aquarium, however, a number of marine fishes have been found to be sensitive to concentrations of less than 0.20 p.p.m. (Herald et al., 1962).

Invertebrates are much more sensitive; for example, sea-urchin larvae were strongly affected by 0.03 p.p.m. of copper (Wilson and Armstrong, 1961). Surprisingly little metallic copper need be exposed to sea water to produce a dangerous concentration of dissolved copper. Braker (1961) placed two U.S. pennies in a system containing 6 gallons of sea water with 1 gallon per hour flowing over the copper coins. He found a concentration of 0.1 p.p.m. of copper in the ambient sea water at the end of 12 hours, and 0.58 p.p.m. at the end of 48. (At this rate, it would take more than 5 years for the coins to dissolve.) We have received several reports that fresh sea water flowing through a single copper, brass, or bronze fixture can pick up enough copper to kill larvae. One reason for the acute sensitivity of fishes and aquatic invertebrates to a heavy metal under these conditions is that they act as biological accumulators, removing the metal from solution and retaining it.

Few determinations of the toxicity of substances in sea water have been made, but materials toxic in fresh water are usually also toxic in sea water, although somewhat less so. Exceptions are known. Boëtius (1960) found that although mercuric chloride is less toxic to small fish in sea water than it is in fresh, phenylmercuric acetate is more toxic in sea water. Certain aquarium cements, whose performance in fresh water is satisfactory, break down in salt water and release substances that kill fish. In general, all metals should be avoided, even those like lead and stainless steel that are sometimes considered safe. Wood and other questionable materials may be covered with several coats of a good grade of black asphaltum varnish, but this protective coating will not last indefinitely and requires periodic checking. Cement is inert, but only after it has been "cured," a process that may take months. Chemically ideal for sea-water systems is hard rubber, also called vulcanite and ebonite, the use of which was first recommended by Michael Faraday in 1857 (Lloyd, 1871). In recent years, a number of plastics have also proved satisfactory. Not all plastics are safe to use, however. In fact, if there is any doubt about the suitability of a material, it ought to be rigorously tested, by the animals themselves, as recommended and described by MacGinitie (1947).

NATURAL CONSTITUENTS OF SEA WATER

Overlying the differences between sea water in nature and in confinement is the basic similarity of its composition the world over. The ratios between the nine ions which constitute more than 99 percent of its dissolved salts are virtually constant (Sverdrup, Johnson, and Fleming, 1942; Harvey, 1955). This is a fortunate circumstance that permits a great deal of uniformity in approach and procedure by aquarists and experimentalists as well as oceanographers. On the other hand, at least 52 elements have been found in natural sea water, and this complexity has led to some irrational ideas about its chemical composition, particularly in connection with its duplication in the laboratory as artificial sea water. A fundamental difficulty is that conventional chemical notation cannot represent a mixture of many anions, cations, and molecules, all in dynamic equilibrium. There is perhaps no more striking illustration of this and its relation to life in the sea than the observation that a volume of sea water allowed to evaporate to dryness and then reconstituted with the proper amount of distilled water will not support the variety of marine life it originally would.

Moreover, as found in nature, sea water is not only an inorganic complex. An ever-increasing number and variety of organic substances are being found in it. Although there is very little organic matter in sea water quantitatively speaking, evidence is accumulating that the little there is exerts an important influence on marine life (Collier, 1953; Lucas, 1955; Nigrelli, 1958). Among the various types of organic substances that have been found in sea water are enzymes, vitamins, pigments, amino acids, antibiotics, and toxins, most of them presumably produced as external metabolites or ectocrines by plants and animals in the sea. The role that such minute constituents of sea water can play in maintaining marine life in captivity has been determined for only a few microorganisms, and their fate, when subjected to the changes that "captive" sea water undergoes, is unknown. Nevertheless, their existence is noted here because of the good possibility that some of them may be important in the culturing of larval forms and macroscopic invertebrates.

Whatever may be their bases, the differences between various natural sea waters can be extraordinarily subtle, as the decade of painstaking work by Wilson and Armstrong has shown. Faced with similar mysteries, aquarists have attributed a sort of *élan vital* to sea water and called it "living"—for instance, when they have observed that artificial sea water had to be inoculated with some of the natural stuff before most invertebrates and some fishes would live in it. Vitalism is not the answer, of course, but there are vital constituents of sea water that are still unrecognized.

CHANGES OCCURRING IN SEA WATER REMOVED FROM THE SEA

Within 30 minutes after a volume of sea water has been taken from the sea, detectable chemical changes have occurred in it (Collier and Marvin, 1953). Biotic activity is responsible for this, the forerunner of a series of profound changes that are brought about in stored sea water by bacteria. Although the sea contains a widely distributed microscopic fauna and flora consisting of protozoans, algae, fungi, yeasts, and bacteria, only bacteria have as yet been found to be important in sea water maintained in vessels or water systems (ZoBell, 1946, 1959; Harvey, 1955). When fresh, filtered sea water is stored, there is a tremendous increase in the number of bacteria, a maximum population of from 2 million to 100 million per cc. being attained in 3 to 6 days at room temperature (ZoBell and Anderson, 1936). In about 2 weeks the initial growth phase is over, and the population then fluctuates from a few thousand to more than 100,000 per cc. At the same time, the number of species of bacteria is drastically reduced to less than half a dozen. Sea water stored for 4 years at 2° to 6° C. still contained more than 200,000 bacteria per cc. (ZoBell and Anderson, 1936), while that stored at room temperature became practically sterile in 6 months (MacGinitie, personal communication).

During the period of the precipitous growth and decline in number of bacteria and for some time thereafter, stored sea water is lethal to the more delicate creatures reared in marine laboratories (MacGinitie, 1947). Because of similar difficulties, aquarists using natural sea water in small aquariums (up to about 100 gallons) subscribe to the procedure of storing the water in glass in the dark for 6 weeks before use. The lethal effect appears to result from the presence of bacterial metabolites, since aeration is not supposed to relieve the condition. In contrast, public aquariums have never reported any difficulties with fresh sea water, perhaps because of the much greater volumes of water, more effective filtration, and lack of delicate forms in their establishments.

The control of bacteria must be one of the principal objectives in the management of salt-water systems and aquariums, but so little is known about the forms that occur in these artificial habitats that any approach is largely empirical. Although sea water has well-recognized bactericidal effects, it also provides an excellent medium for some bacteria, and when enriched with organic matter, may contain billions per cc. These microorganisms can reduce the amount of dissolved oxygen. They can also produce asphyxiating amounts of carbon dioxide and in this way, as well as others, may dangerously lower the pH. Under anaerobic conditions, they produce methane and hydrogen sulphide, the latter of which is known to be strongly toxic to fish (Doudoroff and Katz, 1950). Quite possibly they produce metabolites that are inimical to higher forms. Although this has never been demonstrated, Harvey (1925) found that sea water in which a worm had putrified or to which enough peptone had been added to make it become cloudy was toxic to a species of copepod for some days, even after it had been filtered through a Berkfeldt candle several times and also well aerated.

A *sine qua non* of bacterial control in confined bodies of sea water is cleanliness, achieved by avoiding overfeeding and overcrowding and by practicing systematic but not stereotyped cleaning procedures. In addition, four methods of reducing the number of bacteria have been used, viz., filtration, storage in the dark, ultraviolet treatment, and addition of antibiotics. The efficacy of filtration and storage had been well known long before Stowell and Clancey (1927) found, at the London Aquarium, that storage in the dark for 28 days eliminated “66 per cent. of the ordinary bacteria and 97 per cent. of the so-called ‘blood-heat’ organisms and bacteria of the human intestinal type,” and Lackey (1956) determined that passage through the filters at Marine Studios (Marineland) generally removed more than half of the circulating bacteria. Herald et al. (1962) found that exposure to ultraviolet reduced demonstrable bacterial populations of 1,200 to 2,000 per cc. to practically zero. Marshall and Orr (1958) successfully used chloromycetin and streptomycin to prevent the bacterial multiplication that occurs when fresh sea water is put into aquariums, but there were indications that antibiotics may sometimes harm invertebrates exposed to them.

The importance of microorganisms, especially bacteria, to the state of the water in marine aquariums can scarcely be overemphasized, and not until we have a better understanding of bacterial activities can we hope to develop a truly rational technique of taking care of it. The fundamental difference between the behavior of small standing fresh-water aquariums and those containing sea water lies with their microbiology. The real "balance" of the former consists in the relatively stable condition of its microbial population, even in the presence of quite large amounts of organic material. The basis for this phenomenon is not definitely known. Breder (1931) has suggested that it may be partly the development of buffering capacity by the water and the appearance of bacteriophage, while studies of Dr. Seymour Hutner have indicated that the development of a dominant population of predatory protozoans is largely responsible. The situation in fresh water may well be exceptional, but the reasons for its absence in sea water would nevertheless be of interest. For example, bacteriophages have been found in the ocean (Spencer, 1960), but they do not seem to have been looked for in marine aquariums or other artificial environments.

CHANGES OCCURRING IN SEA WATER AFTER LONG USE

The outstanding changes occurring in sea water that has been kept in circulatory systems for months or years are two: a permanent lowering of the pH and an accumulation of nitrates. After 20 years of use, an analysis of the sea water in the New York Aquarium revealed that the nitrate content had increased more than 250-fold and that the pH had dropped below neutrality (Townsend, 1928). Similar data, involving similar or shorter periods of time, have been reported for the London Aquarium (Stowell, 1926a; Brown, 1929; Oliver, 1957), the Amsterdam Aquarium (Honig, 1934; Sunier, 1951), and the Ueno Aquarium (Saeki, 1958).

In nature, the pH of sea water seldom exceeds 8.4 or drops below 7.5 (Sverdrup, Johnson, and Fleming, 1942). It is regulated by a series of chemical equilibria involving carbon dioxide, carbonic acid, sodium bicarbonate, and sodium carbonate which forms a buffer mechanism and makes possible the addition of relatively large amounts of acid or alkali before the pH is appreciably altered (Harvey, 1928, 1955). If enough acid is added, all the excess base (alkali reserve) will be destroyed, but this occurs at a pH of 5.5, a far stronger acidity than the vast majority of marine fishes and invertebrates can endure. There has been disagreement as to the exact source of the acidity brought about by the metabolism of fish, in addition to that caused by the carbon dioxide they produce, but it is agreed that oxidation is the ultimate fate of practically all the excretions of fish— which is, of course, an acid-producing process.

Three methods of maintaining the pH in marine circulations have been used. At the Plymouth Aquarium, freshly slaked lime ($Ca(OH)_2$) is periodically added to the reservoir (Brown, 1929; Atkins, 1931; Wilson, 1952, 1960). At the New York Aquarium, sodium bicarbonate ($NaHCO_3$) was added continuously to the circulating water (Breder and Howley, 1931), and modifications of this procedure have been used by other aquariums including the John G. Shedd Aquarium and the London Aquarium. At the Amsterdam, London, Ueno, and New York Aquariums, quantities of some form of calcium carbonate ($CaCO_3$) such as bivalve shells, marble chips, coral sand, or calcite, are kept in contact with the circulating water (Sunier, 1951; Oliver, 1957; Saeki, 1958). The relative merits of treatment with slaked lime or sodium bicarbonate were briefly argued by Breder and Howley (1931), Atkins (1931), Breder and Smith (1932), and Cooper (1932). One noteworthy aspect of this is the great importance that Breder (1934) attributed to the calcium ion in keeping captive marine fishes.

Although there are differences of opinion whether ammonia is the characteristic excretory product of aquatic organisms (Smith, 1953), it is agreed that this substance is at least one of the principal waste products of fish and aquatic invertebrates. It is also the main nitrogenous substance resulting from the bacterial decomposition of plant and animal tissues under both aerobic and anaerobic conditions in sea water (ZoBell, 1959). Since ammonia is highly toxic to fish in fresh waters, particularly alkaline ones (Doudoroff and Katz, 1950), and there is every reason to believe that it is comparably toxic in sea water, the fate of this metabolite is of considerable concern to the marine aquarist. Some ammonia passes into the atmosphere; the remainder, which is undoubtedly the bulk of it, is oxidized by bacteria to nitrites and nitrates. Large numbers of these bacteria are found in aquarium filters, but some also live on the walls of the tanks (Saeki, 1958). In a quantitative experiment concerning the nitrogenous substances in closed sea-water circulations, Saeki (1958) found that about 25 percent of the decrease in ammonia resulted from oxidation in the filter by its *Schmutzdecke*. Storage of aquarium water in the dark also reduces its ammonia content (Stowell, 1926b). The latter may be the result of direct chemical oxidation as well as biotic activity (Harvey, 1955; Vaccaro, 1962). There are bacteria that reduce nitrates and nitrites to nitrogen and others that reduce nitrates to nitrites, but what significant part, if any, they play in the nitrogen cycle of aquariums is unknown.

In fresh water, nitrates are hardly more toxic to fish than chlorides (Trama, 1954), and no adverse effects on marine species have ever been reported. According to Oliver (1957), however, nitrate can interfere with the respiratory processes of some animals, in particular marine invertebrates. Effects of nitrite do not ever seem to have been described. Oliver (1957) has pointed out that there is no chemical way of removing nitrate, and to accomplish this some adaptation of the denitrifying activities of bacteria appears to offer the most promise. Indeed, Honig (1934) was able practically to eliminate nitrite and nitrate in an experimental marine system by encouraging the multiplication of denitrifying bacteria, but Sunier (1951) reported that the practical application of her method presented difficulties. In a 6,600-gallon sea-water system at the Wuppertal zoological gardens, the combination of filtration by a pressure filter charged with activated carbon and a specially encouraged growth of marine algae kept nitrite and nitrate concentrations within the range of natural sea water (Seiffge, 1941).

The reduction of nitrate and nitrite to ammonia and the formation of ammonia from bacterial decomposition of proteinaceous substances causes an increase in the pH of sea water, while bacterial oxidation of ammonia to nitrite or nitrate causes a decrease (ZoBell, 1959). These relationships require consideration, for Saeki (1958) has reported that a low pH inhibits the oxidation of ammonia in aquariums.

DISSOLVED RESPIRATORY GASES

Sea water can dissolve about 20 percent less oxygen than fresh water; at 20° C., for example, oxygen-saturated sea water (salinity 36 parts per thousand) contains 7.12 p.p.m. of that gas, as compared with 8.84 for distilled water (Truesdale, Downing, and Lowden, 1955). A survey of the literature has shown that the minimum oxygen requirements of marine fishes range from 3.3 to 0.1 p.p.m. At the London and Plymouth aquariums, Brown (1929) found that the sea-water systems were 60 to 90 percent saturated with oxygen. No temperatures are given, but at 20° C., this would correspond to at least 4 p.p.m., and at 30° C., to 3.6 p.p.m., which may be considered adequate for the majority of fishes.

The carbon dioxide content of sea water influences the respiration of fish by reducing the amount of oxygen carried by their red blood corpuscles (Fry, 1957). This is also a pH effect, and it would therefore be expected that sea water whose pH was low—whether from an accumulation of carbon dioxide or a loss of alkali reserve—would inflict respiratory stress on various fishes. Although general aquarium experience bears this out, no experiments to demonstrate the phenomenon have ever been reported. A pH of 7.8 or more appears to be safe for all fishes, while one of 7.0, or even 6.8, is adequate for some of them (Oliver, 1957). One important characteristic of carbon dioxide and its passage between the sea and the atmosphere is that the process takes place relatively slowly, and even with artificial aeration may require several hours (Harvey, 1928, 1955).

Marine fishes that are exposed to sea water supersaturated with air frequently develop gas-bubble disease in which bubbles, mostly of nitrogen, appear under the skin, especially on the fins, and within various vital organs, causing exophthalmia, loss of equilibrium, and death. The supersaturation generally results from a leaky pump or pipeline which permits air to enter and mix with water that is put under pressure, or from a sudden, relatively great increase in water temperature. Marsh and Gorham (1905) first described the disease and its cause in detail, and their account was based on its occurrence in fishes living in a sea-water circulatory system. Since then, all investigations appear to have been carried out on fresh-water species (Harvey and Smith, 1961). Dr. Ross F. Nigrelli, Pathologist of the New York Aquarium, has suggested that denitrifying bacteria may be a source of nitrogen that causes gas-bubble disease.

For the aquarist or experimentalist with a physicochemical background, the papers by Downing and Truesdale (1955, 1956) and Haney (1954) provide basic and theoretical data on the aeration of aquariums.

MARINE CIRCULATORY SYSTEMS

From the preceding account, there can be little doubt of the complexity of interrelations between captive marine macro- and micro-organisms and the sea water in which they live (see fig. 1). Early aquarists considered their aquariums and water systems to be microcosms whose elements behave in the same way as they do in the whole earth's grand economy (e.g., Lankester, 1856; Mather, 1880), and this attractive but erroneous idea has persisted for many years (see Stowell, 1926a). As a consequence, the early aquarists who discovered such vital techniques as circulating the sea water, storing it in the dark, and aerating it, attributed their usefulness to quite incorrect reasons (Lloyd, 1871; Newman, 1873). Standing on the shoulders of these pioneers, we now recognize many significant differences between the bionomics of aquarium systems and the sea.

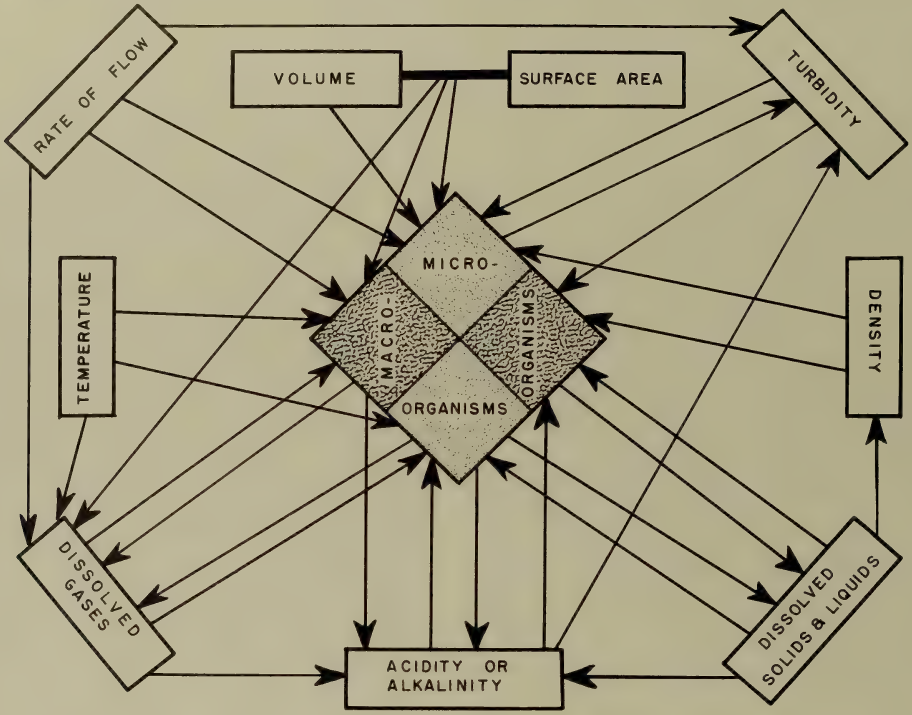

Figure 1.—Diagram of the complex interactions of macroorganisms and microorganisms and environment in a marine aquarium or sea-water system. Arrows point from the agent or influencing factor to the item changed or influenced.

The need for constant treatment of sea water in order to keep it satisfactory for marine life is apparent. At the very least, sea water must be aerated continuously, but unless it is to be replaced often and unless the ratio of volume of water to volume of animals living in it is extremely large, circulation, filtration, and storage in some sort of reservoir are also necessary. In a typical circulatory system, the water flows from tank (exhibition, holding, or experimental) to filter, to reservoir, and back to tank (see fig. 2). Aerators are frequently located between each of these units and the next. The pump is placed after the reservoir because it is least likely to become clogged with animals, sand, or solid wastes at this spot. A gravity tank greatly facilitates the regulation of water flow into the animals' tanks and lessens the chance of introducing water supersaturated with air.

Systems in which the water is recirculated, being used over and over, are called

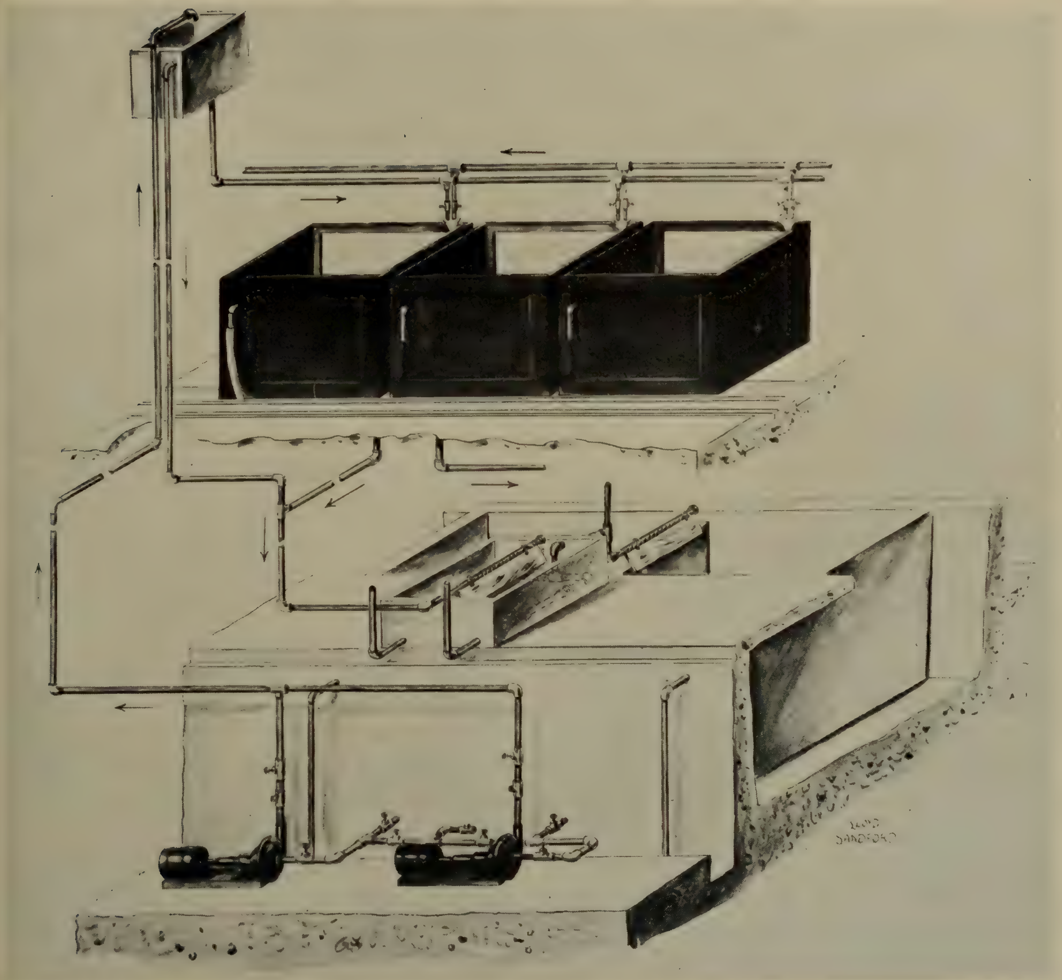

FIGURE 2.—Typical closed sea-water circulatory system. The water flows as follows: Holding tank (black) to return trough and pipeline, to manifold aerator, to filter, to manifold aerator, to reservoir, to pump, to supply riser, to gravity tank, to supply pipeline, to holding tank. Note that all service units, except the gravity tank, are in duplicate and that valves are arranged to provide complete flexibility—that is, all possible combinations of units. The holding tanks are connected to two supply pipelines, and a length of flexible hose from each overflow makes it possible to use either return trough.

"closed." A typical "open" sea-water system has been described by Hinton (1958). In this, the water is used once and then discarded. The tanks of such a system should be connected in parallel to the water source, not in series, so that each tank receives the same kind of water, and disease cannot be spread through the water from tank to tank. Both Hinton (1958) and MacGinitie (1947) point out that the storage tank, which provides an emergency supply of water if the pumps fail and also acts as a settling tank, should not contain more than a 36-hour supply and that a 24-hour supply is usually sufficient.

In order to prevent the spread of disease and facilitate water treatment, each tank should have its own circulatory system with an individual pump, filter, and reservoir, but the expense of initial installation and of maintenance may preclude this ideal arrangement, especially if large tanks are involved. When the units have been small enough (less than 1,000 gallons) that they could be provided adequate circulations by means of air lifts, individual sea-water circulatory systems have been set up in public aquariums, for example, at Wuppertal (Wiedemann, 1943) and Bern (Hediger, 1944). Ingenious modifications of filters and aquariums making them more suitable for individual circulations have been described by Wiedemann, by Chin (1959), and by Kelley and Moreno (1961).

In some sea-water systems, there is no filter; instead the water is allowed to pass slowly through a large settling tank or reservoir. Herald et al. (1962) have compared this type with the one using filtration, and they indicate that the latter carries a greater proportion of animals, viz., about 50 gallons of sea water per pound of living animals as compared with about 450 gallons for a system depending on sedimentation alone. Wilson (1952, 1960) has described a typical filterless system. In his later paper, he stresses the importance of having a reservoir that is correctly designed, and he provides the details of a successful one now in use at the Plymouth Aquarium. One advantage of sedimentation over filtration is that filter-feeding invertebrates do better in unfiltered water (Wilson, 1952).

Filtering tests that were made on beds consisting of uniform-sized silica sand by William E. Kelley of the Cleveland Aquarium (unpublished report) and of a graded series of quartz or quartzlike filtrants by Herald et al. (1962) showed that practically all of the filtering was accomplished in the top few inches of the bed. Conventional deep filters would therefore appear not to be necessary in sea-water systems. Saeki (1958), however, has determined the amount of sand that will provide space for the bacteria necessary to oxidize the waste products of a fish, and he states that as a minimum the filtrant should weigh 30 times as much as the fish. Evidently volume as well as surface should be considered in calculating filter requirements. An example of a shallow-bed filter that uses a uniformly sized filtrant is shown in figure 3.

Hinton (1958) has discussed the problem of an optimum rate of flow in a sea-water circulation and concludes that it is difficult to have too much sea water. The

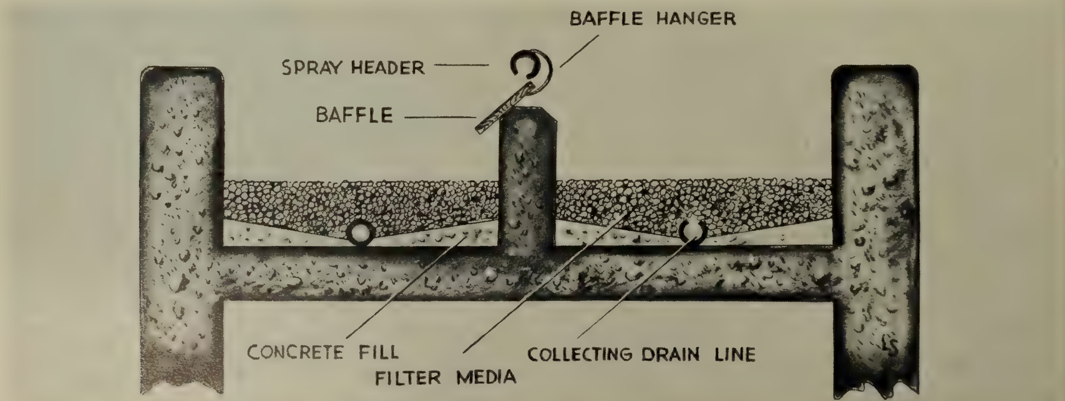

FIGURE 3.—Cross section of shallow-bed filter seen in figure 2. The spray header acts as a manifold aerator, and the baffle can be reversed to direct the water into the opposite filter chamber. Backwashing water is introduced into the collecting drain line through its elevated open end (seen in figure 2).

water in the tanks of the aquarium at the Scripps Institution of Oceanography is changed once every 3 to 5 hours, and this has proved satisfactory. At the Plymouth Aquarium, however, a turnover of slightly more than four times a day was barely adequate (Wilson, 1960). At the Amsterdam Aquarium, an entirely different situation prevails, with the water in each tank being replaced on an average of only 1½ times a day (Sunier, 1951). We believe that for most laboratories and aquariums, an ideal rate would be 20 to 24 changes of water a day (see Addendum).

A cardinal, though unproven, principle of sea-water management is that the greater the volume, the slower its rate of deterioration. General aquarium experience, without any doubt, abundantly supports the corollary principle that the ratio of volume of sea water to volume of animals kept in it should be as large as possible. Wilson (1952) has reported an informal experiment that was dictated by World War II at the Plymouth Aquarium: "During the war when most of our big tanks were broken and empty, the inhabitants of those which remained did noticeably better, and delicate organisms survived longer than they did before the war, or do now, and sometimes even bred." The greatest ratio ever maintained in a large sea-water circulation seems to be that in the Amsterdam Aquarium, where the tanks containing the marine animals comprise but one sixth of the total volume of the system. One way of lengthening the useful life of a volume of sea water is to divide it into two equal parts and alternatively "rest" half of the water in a dark reservoir, usually for a month or 6 weeks (Stowell and Clancey, 1927; Wilson, 1952, 1960).

ADDENDUM

On the basis of the work of Saeki (1958) and experiments recently performed at the Cleveland Aquarium, Kelley (1963) has described the parameters of an ideal sea-water circulatory system. The relation of volume of water to weight of animals maintained in it is 100 gallons to 1 pound. The water circulates completely once every hour and passes through the filter at a rate of 1 gallon per square foot per minute. The filtrant consists of 2- to 5-mm. grains of silica gravel (75 percent) and calcareous gravel (25 percent) and there is 1 cubic foot of it for each pound of animal.

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A NEW FORMULA FOR ARTIFICIAL SEA WATER

By Richard Segedi and William E. Kelley
The Cleveland Aquarium, Cleveland, Ohio

Abstract.—An artificial substitute for sea water is offered which has performed well in keeping both vertebrates and invertebrates at the Cleveland Aquarium. The formula is assembled and stored for convenient use as a 4-part preparation.

The use of artificial sea water is nearly as old as the keeping of marine aquariums. In 1854, Gosse, who was one of the principal popularizers of the home aquarium, both fresh-water and marine, described his experiences with simple mixtures of readily available chemicals that could be used when natural sea water proved too difficult to procure.

This was hardly a year after the opening of the world's first public aquarium in London. A few unsuccessful experiments with synthetic sea water were subsequently carried out in some of the early aquariums, but the first institution to use it with any success appears to have been the Berlin Aquarium, which opened to the public in 1869. The recipe was a simple one, involving only four salts, and, like the mixtures concocted by Gosse, it required an inoculation of seaweed or some similar living organisms to make it fit for higher forms of marine life (Hoffmann, 1884). If the great success claimed for this *Ersatzseewasser* was not at all exaggerated, it is difficult to find any reason why most aquariums did not evenutally adopt it. However, very few institutions have found artificial sea water to be a satisfactory substitute for the natural product, one noteworthy exception being another Berlin aquarium, which opened to the public in 1913, as described by Heinroth (1937).

Several different formulas for artificial sea water have been devised (table 1). The number of ingredients in them varies from 4 to 14, but the avowed purpose of them all was to provide as close a facsimile as possible to actual sea water.

THE BACKHAUS FORMULA

In December 1960, one of us (Kelley) visited the Exotarium of the zoological gardens of Frankfurt am Main, and while there was most favorably impressed with both the practical and the theoretical aspects of the synthetic sea water that had been prepared by Dr. Dieter Backhaus of that institution. Dr. Backhaus' approach to the problem had not been to try to imitate natural sea water, but to produce the artificial medium in which marine animals would thrive best, regardless of how closely it might or might not resemble the composition of sea water in nature. It was recognized that the synthetic mixture would have to resemble natural sea water at least in the rough proportions of its principal salts, but these were only taken as a general guide in the first formulations. The appearance and behavior of the many marine fishes and invertebrates on exhibition at the Exotarium attested to the soundness of the new approach, and Dr. Backhaus graciously made his formula available to us for further experiment.

We have modified the Backhaus formula for artificial sea water into a 4-step preparation as shown below (for preparing 100 gallons with specific gravity of 1.025):

PART I

$NaCl$	10.43	kg.	Technical grade.
$MgCl_2.6H_2O$	2.04	kg.	Do.
$MgSO_4.7H_2O$	2.62	kg.	Do.
KCl	277.8	g.	Do.
$NaHCO_3$	79.4	g.	Do.
$SrCl_2.6H_2O$	7.5	g.	Analytical reagent.
$Mn(SO_4)_2$	1.5	g.	Do.
$Na_2HPO_4.\ 7H_2O$	1.25	g.	Do.
$LiCl$	.375	g.	Do.
$Na_2MoO_4.\ 2H_2O$	.375	g.	Do.

PART II

$CaCl_2$	522	g.	Technical grade.

Trace Element Stock Solutions

Add 80 cc. each of solutions III and IV.

PART III

Calcium Gluconate	6.25	g.	U.S.P.
KI	.9	g.	Analytical reagent.
KBr	270.00	g.	Do.
$CuSO_4.\ 5H_2O$	4.3	g.	Do.

Dissolve in 2 liters of distilled water.

PART IV

$Al_2(SO_4)_3$	4.5	g.	Analytical reagent.
$CoSO_4$	.5	g.	Feed grade.
$RbCl$	1.5	g.	Analytical reagent.
$ZnSO_4.\ 7H_2O$	.96	g.	Do.

Dissolve in 2 liters of distilled water.

We have also altered a few of the proportions of its constituents:

1. The Backhaus formula includes slightly more than a millionth part of iron, which we have omitted because our Great Lakes tap water is relatively rich in this element.

2. The Backhaus formula calls for about one five-thousandth part of zinc sulfate and one fifty-thousandth part of copper sulfate. Fearing synergistic toxicity, we have reduced these to the amounts found in natural sea water.

3. We have eliminated the boric acid used by Dr. Backhaus to inhibit plant growth in amounts up to six ten-thousandth parts.

We have not found it necessary to use chemically pure (CP) substances throughout as did Dr. Backhaus.

Part I of the formula consists of its gross dry components, and these may be weighed and placed in a mixing tank. A hard stream of water directed into them will dissolve these chemicals. The container may then be filled almost to the level of the desired specific gravity.

Part II, which consists of the calcium chloride, is dissolved in hot water and added to the mix. Additional water may then be added to bring the mix to the correct specific gravity. Calcium chloride is mixed separately because, at the high concentrations of the gross components, it will react with the magnesium sulfate to form magnesium chloride and calcium sulfate, the latter of which will precipitate.

Parts III and IV, which are stock solutions of trace elements, may now be added to the mix. Liquid stock solutions of these are desirable because (1) the assembly of the parts is made more convenient, (2) some of the compounds providing trace elements must be dissolved by heat (calcium gluconate, cobalt sulfate, and aluminum sulfate), and (3) better quantitative accuracy may be achieved by weighing out relatively large amounts for the stock solutions. If chlorinated tap water has been used, the solution must be aerated for a day or two before adding parts III and IV, or the residual chlorine will displace the ionic bromine and iodine since the latter occupy a lower position in the electromotive series.

We have been able to maintain marine invertebrates indefinitely in this artificial sea water—something we have not been able to do with any other type.

A summary of references to various types of artificial sea water is given in the following listing:

Originator	*Reference*
Hermes, ca. 1880	Hoffmann (1884).
Schmalz, ca. 1913	Heinroth (1937), Needham et al. (1937), Hediger (1944).
Von Flack	Needham et al. (1937).
McClendon et al., 1917.	Needham et al. (1937), Sverdrup et al. (1942), Redfield (1948), Getchell (1953).
Brujewicz (in Subow, 1931).	Sverdrup et al. (1942), Redfield (1948), Getchell (1953).
Penn, A.B.K., 1934.	Needham et al. (1937).
Lyman and Fleming, 1940.	Sverdrup et al. (1942), Redfield (1948), Getchell (1953), Harvey (1955).
Dählhölzli	Hediger (1944).
U.S. Navy corrosion test. Aquarium.	Redfield (1948).
John G. Shedd Aquarium.	Getchell (1953).
Wilder, 1953	Pachtman (1956).

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NOTES FROM THE AQUARIUM OF THE MARINE LABORATORY, ABERDEEN

By H. J. Thomas

Department of Agriculture and Fisheries for Scotland, Marine Laboratory, Aberdeen, Scotland

Abstract.—The closed circulation system described comprises twin reservoir tanks, service tank, header tank, display tanks, and filter bed. Special features include construction in water-tightened concrete with vulcanite-lined cast-iron or alkathene tubing, reservoir tank capacity five times that of the display tanks, aerator pump which is water sealed, and the use of marble chips in the filter bed. The aquarium is furnished with several levels of temperature control to allow maintenance of the appropriate seasonal sea temperature. Isolation tanks are provided for experiments which might involve risks if undertaken in the main system.

The Marine Laboratory's aquarium is essentially a research tool, but the basic design, stocking, and temporary decoration of the tanks is adapted to afford as high a standard for display as is compatible with the primary function.

STRUCTURAL DATA

The basic layout follows the standard pattern for a closed-circulation sea-water aquarium (figs. 1 and 2). The relevant capacities and ratings of the main aquarium installations are as follows:

Volume of large display tanks (9 at 5 by 5 by 5 feet)	7,000 gallons.
Volume of small display tanks (8 at 3 by 2½ by 2½ feet)	800 gallons.
Volume of portable tanks (as required)	400 gallons.
Total volume of display tanks	8,000 gallons.
Volume of service tank	500 gallons.
Volume of header tank	350 gallons.
Reservoir/sedimentation twin tanks, each of capacity	40,000 gallons.
Main circulation pump rated capacity	4,000 gallons per hour.
Filter bed, combined total filter area	12 by 16 feet.
Refrigerator rated capacity	60,000 B.t.u.
Heater capacity	3 kilowatts.

Reinforced, water-tightened, sulphate-resisting, concrete units are poured in a single operation, thereby avoiding sutures with consequent corrosion of the reinforcing by sea water. In the reservoir tanks where single-operation pouring is difficult, the outer reinforced-concrete shell is lined with asphalt, and this in turn is supported by a brick layer internally faced with water-tightened concrete. The reservoir tanks are constructed with a sump to facilitate pumping out.

Display-tank walls are smoothed, and all corners are rounded.

One-inch-thick plate-glass fronts to the display tanks are, at their deepest, 4 feet 6 inches from the water surface. Glasses are bonded into position with Glasticon cement of the grade appropriate to the area of the bonding surface, which must be true, and to the depth of the tank.

The original sea-water piping is of vulcanite-lined cast iron. Extensions and replacements are undertaken using alkathene (Imperial Chemical Industries—I.C.I.—black polyethylene).

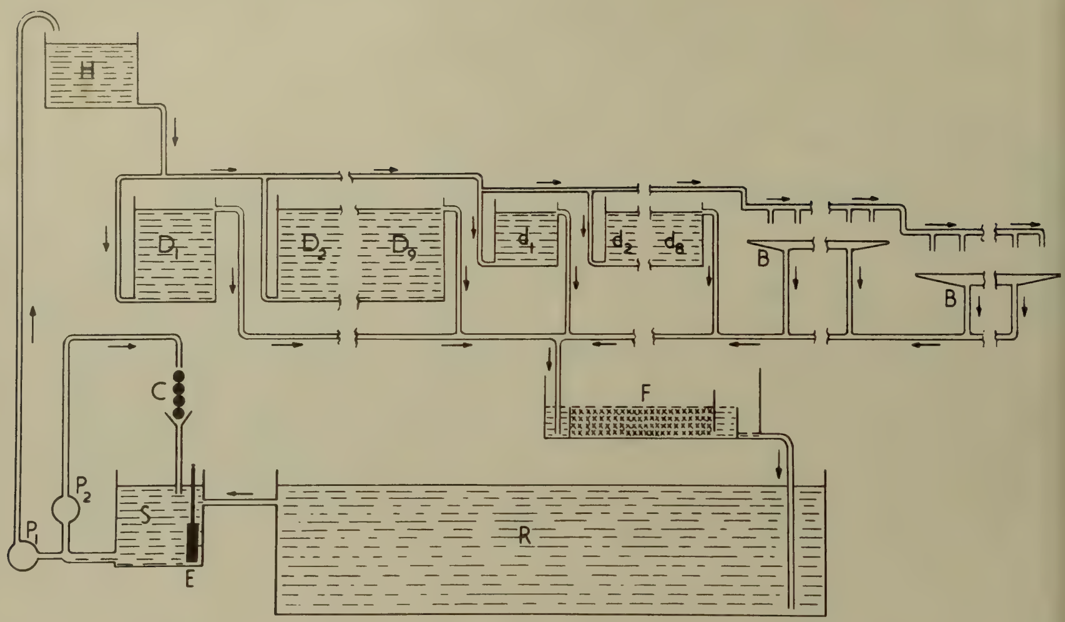

FIGURE 1.—Circulation system of the main aquarium: *B*, experimental benches (high and low); *C*, cooling coils; D_1 to D_9, large display tanks; d_1 to d_8, small display tanks; *E*, immersion heater; *F*, filter; *H*, header tank; P_1, main circulation pump; P_2, cooling circulation pump; *R*, reservoir-sedimentation tank (duplicated); *S*, service tank.

Valves are of the rubber diaphragm type with vulcanite linings (Dexine Rubber & Ebonite Ltd.).

Cut-in/cut-out electrical probes have carbon contacts (stainless-steel probes were found to be unsatisfactory) connected to relays (Sunvic Controls Ltd.).

Air at 10 to 14 pounds' pressure is supplied by a water-sealed compressor (Nash Engineering Company). This avoids oil droplets in the air compressed by oil-sealed pumps. A trap fitted with a float valve collects and drains off the fresh water arising from droplets carried over with the compressed air. Aeration facilities are provided to each tank, but their use is exceptional where tanks are on circulation.

OPERATING DATA

The twin reservoir-sedimentation tanks, each of 40,000 gallons' capacity, are used in the circulation on alternate weeks.

Sea water is added to the system to make up for losses in siphoning and maintenance; to provide the maximum refreshing, any surplus water is run off so that an addition of clean sea water can be made at the rate of 4,000 gallons monthly. Incoming water is added to the tank not on circulation, thereby allowing a period for seasoning. Sea water is transported by lorry in a collapsible Portolite container (Marston Excelsior Ltd., I.C.I.).

The sand filter bed comprising twin sections is wholly in use except for the shutting off of a half section for periodic maintenance. This procedure maintains the filters in a much better condition than alternate use. The pattern of the filter is that normal for domestic fresh-water supplies, but incorporated in the bottom of the filter is a layer of coarse marble chips. This is considered to be responsible for the marked stability of the pH in the aquarium, which has constantly remained

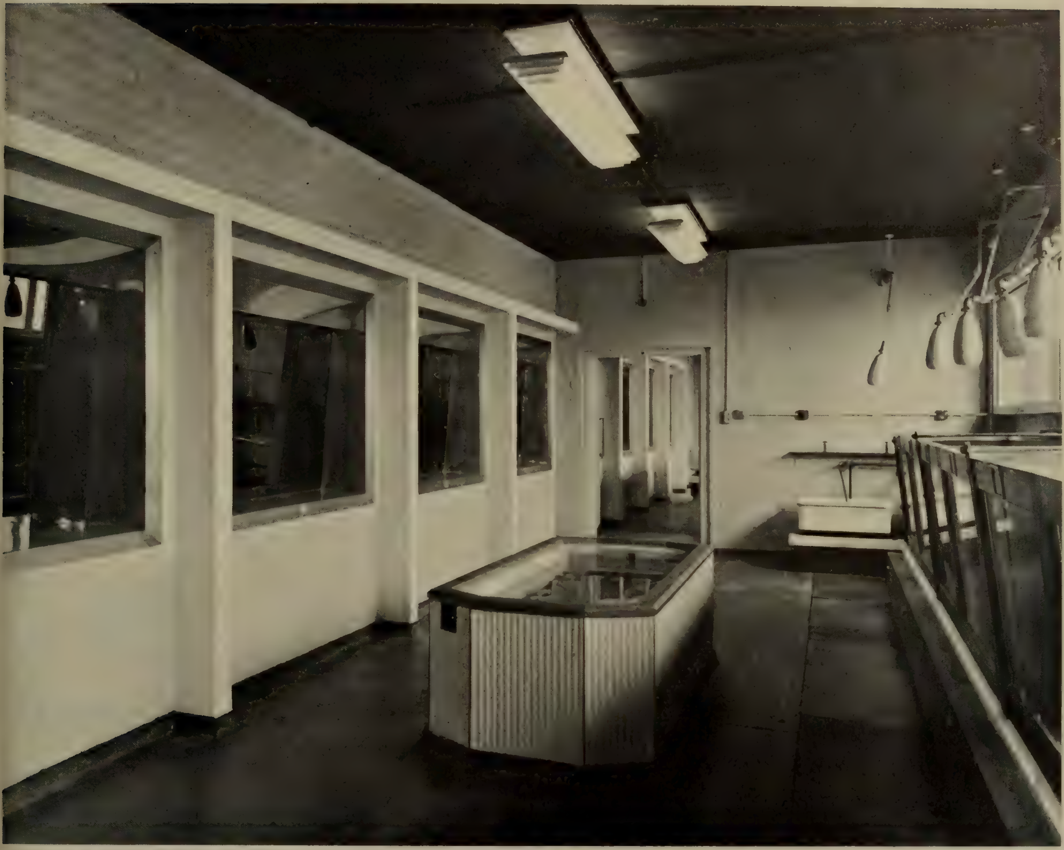

FIGURE 2.—Semipublic part of the aquarium. On the left are the large built-in display tanks illustrated in figure 1, D_1 to D_5; D_6 to D_9 can be seen through the door into the experimental aquarium. Access to these aquariums is by a servicing platform behind the tanks. The center tank is purely decorative. The small display tanks, d_1 to d_8, are on the right.

within the limits normal for water in the open sea. Incorporation of marble has not resulted in any marked increase in the calcium content of the sea water, which is around 425 mg. per liter.

Salinity has remained at around 34 parts per thousand and there has been no occasion to compensate for evaporation.

With few exceptions food is restricted to squid cut to a suitable size and washed. This food has the advantage of being firm, and its use reduces to a minimum the water-soluble and particulate matter which is not taken up by the aquarium animals. Unvaried, it appears to afford a satisfactory diet.

Live material is transported in a carrying tank designed to restrict violent movements of water (fig. 3). When the tank is stocked for carrying, the domed cover is secured in position, and water is added to bring the surface level into the narrow chimney.

Each tank of the aquarium can be isolated from circulation and in these circumstances is furnished with a small portable filter. Sea water is maintained in circulation over this filter by means of an air lift (see also Isolated Tanks, page 25).

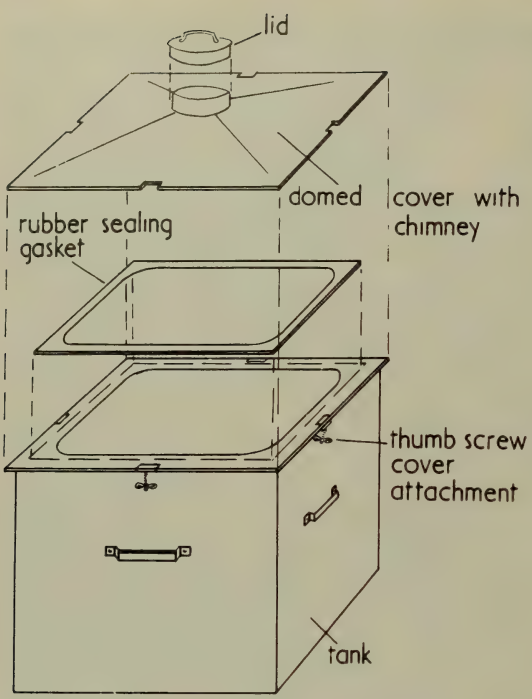

FIGURE 3.—Carrying tank for live material.

TEMPERATURE CONTROL

For general purposes the aquarium water is allowed to take up its natural temperature. In hot weather, however, the maximum temperature is held to about 14° C., whilst in cold weather a minimum of around 6° C. is maintained.

Heating or cooling under thermostatic control is applied in the service tank (fig. 1) which is effectively that section of the reservoir tank from which sea water is immediately drawn to supply the aquarium system. The cooling system is rated to maintain a maximum temperature of 12° C. The heating system is capable of holding 8° C. minimum temperature throughout the aquarium. This allows for the aquarium being run throughout the year at the appropriate seasonal temperature normal to the open sea around Scotland. Alternatively, most of the year, the aquarium can be run at any desired normal temperature between 5° and 16° C. In extremes of climate, such a desired temperature can be held over a part of the aquarium system with the remainder off circulation.

SPECIAL FEATURES

Temperature control is the most frequent requirement in an experimental aquarium. In addition to the general control outlined above, two tanks, each of 700 gallons' capacity, which are normally incorporated in the general circulation, can be isolated, either singly or together, into a separate unit controllable as to temperature within the range 4°–20° C. (figs. 4 and 5). The system alternatively provides an offtake of temperature-controlled sea water to the circulation bench for use with portable tanks. The relevant capacities and ratings for the temperature-controlled system are as follows:

Volume of twin display tanks (700 gallons each)	1,400 gallons
Volume of header heat-exchanger tank	100 gallons
Circulation pump rated capacity	1,000 gallons per hour
Refrigerator rated capacity	27,000 Btu
Heater capacity	6 kilowatts

Experimental procedures frequently demand a sea-water tank controlled to a temperature below room temperature. A small unit (preferably transportable) used for cooling a number of small tanks to different preset temperatures is shown in figure 6. A reservoir of brine, or antifreeze solution, is maintained at a low temperature, and this is pumped through glass cooling coils immersed in the water of the experimental tank(s). The operation of the pump is thermostatically controlled. The number of tanks which can be coupled to this system depends on the regulated temperatures to be maintained. It can be materially increased by insulating the experimental tanks against heat loss.

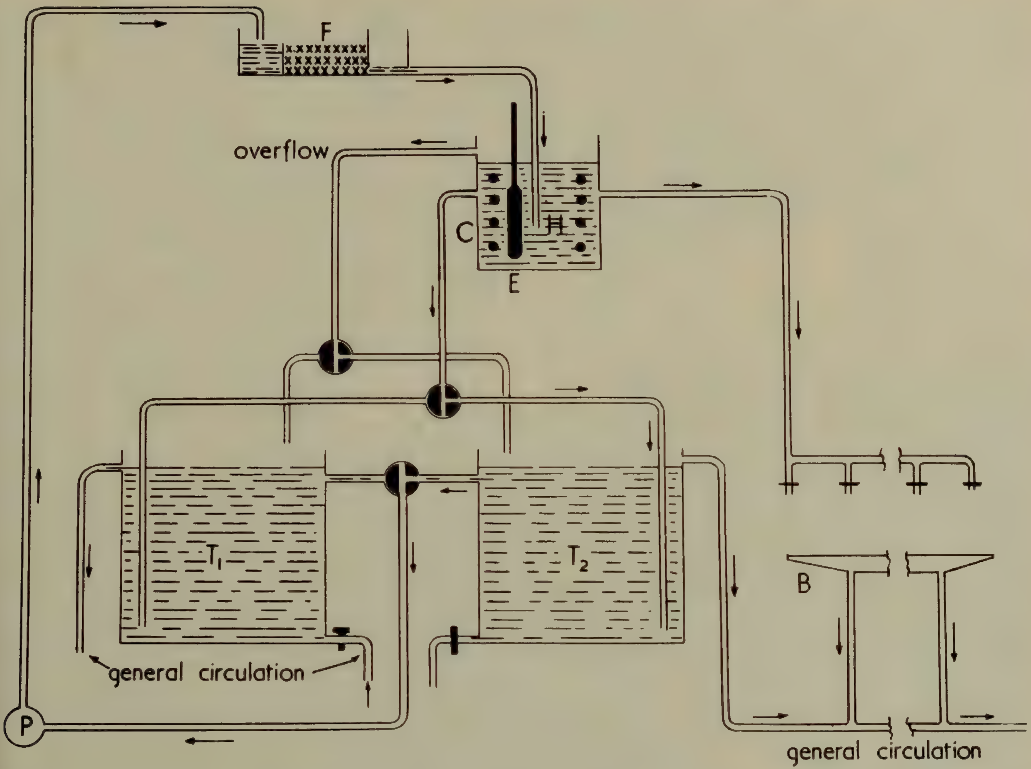

FIGURE 4.—Circulation system of the temperature-regulated experimental tanks: *B*, experimental bench; *C*, cooling coils; *E*, immersion heater; *F*, filter; *H*, header and temperature-control tank; *P*, circulation pump; T_1, experimental tank, depicted on general circulation; T_2, experimental tank, depicted on temperature-control circulation.

ISOLATED TANKS

Experiments frequently involve diseased fish or techniques which may contaminate the sea water. Such experiments must be undertaken in such a way that there can be no risk to the general aquarium circulation, namely, in a separate sea-water tank system. The unit described below has proved most serviceable and consequently is preferred even where the procedures involved are no bar to the use of the main aquarium. The system is simple and relatively cheap to construct.

The unit consists of two reinforced-concrete tanks 12 by 6 by 6 feet, each capable of subdivision by a sectional teak partition (fig. 7). An air lift maintains circulation of the sea water over a sand filter, whence it gravitates back to the bottom of the experimental tank. The base of the filter is at the level of the top of the tank so that the lift is a minimum. Aeration is provided using two Doulton filter candles (Doulton & Co. Ltd.), grade KF, as atomizers. The constructional details follow those outlined for the main aquarium. The tanks and filters are unhoused and fitted with removable covers.

GENERAL

It frequently happens that when an aquarium is in design there are a number of research projects immediately envisaged. Nevertheless it is essential that the

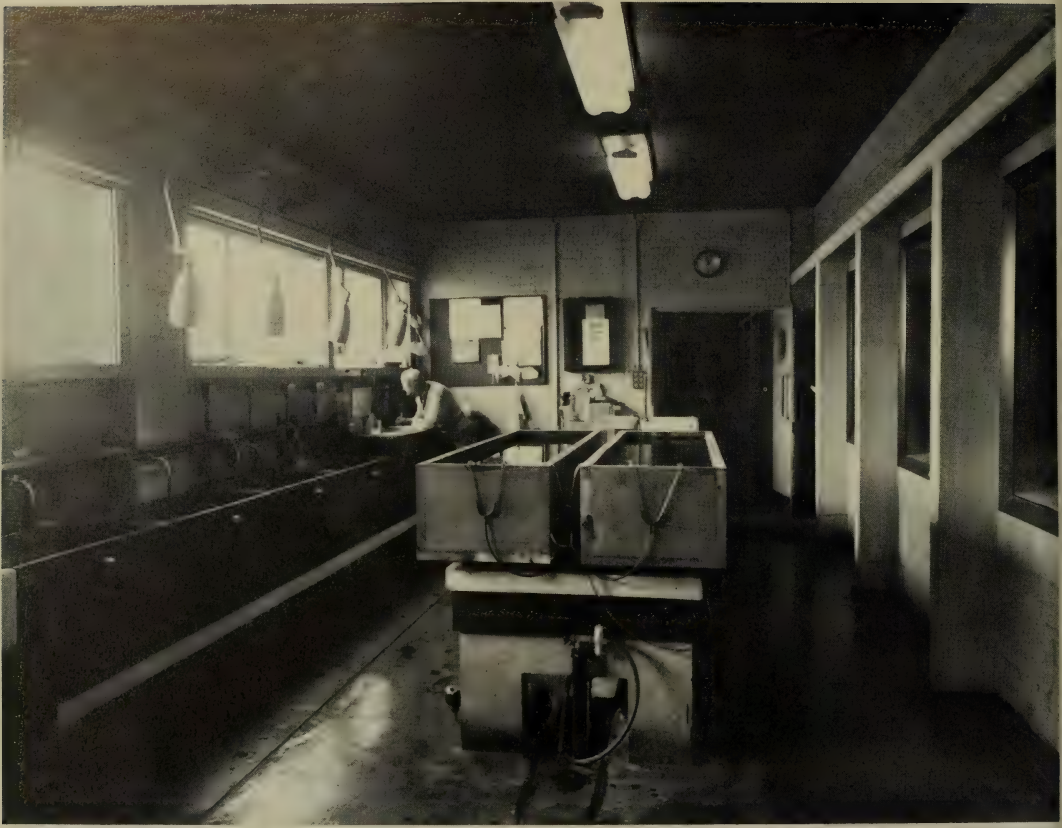

FIGURE 5.—Experimental aquarium. On the left is the low experimental bench; the high bench is in the center. Our aquarium assistant is seen working at a teak-topped bench fitted with hot and cold fresh water and sea water, all running to waste.

basic design be kept simple so as to be as flexible as possible. In this way the inevitable misconceptions in the original research projects will be most readily remedied and thereafter the system be adaptable to the widest possible variety of conditions and requirements.

Successful operation of an experimental aquarium depends upon regular inspection and maintenance of the supply system. We have found this to be greatly facilitated by using standard checklists. Those developed for use with our system are shown below.[1]

DETAILS OF AQUARIUM MAINTENANCE

Daily maintenance

1. Change over all duplicated pump motors (three) and check all other pumps and refrigerator motors in use (12) functionally.
2. Remove dead animals from tanks.
3. Feed aquarium animals as necessary.
4. Check daily diary for experiments being initiated or completed and for arrival of livestock etc., taking the necessary action.
5. Check functionally the aerators in use (two) and the air pressures.
6. Check each tank, including isolated units, for (*a*) air supply, (*b*) level of water, (*c*) rate of circulation, (*d*) cleanliness of the water, and (*e*) cleanliness of the overflow pipe.
7. Check temperatures as shown by recorders (two) and thermostatic control of the system.
8. Check functionally the refrigerator units in use (three), including blower motor and pres-

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[1] Editor's note: These check lists, furnished incidentally by the author for our edification, are included here because we believe that such working details should have great interest to those charged with the day-to-day operation of sea-water supply systems.

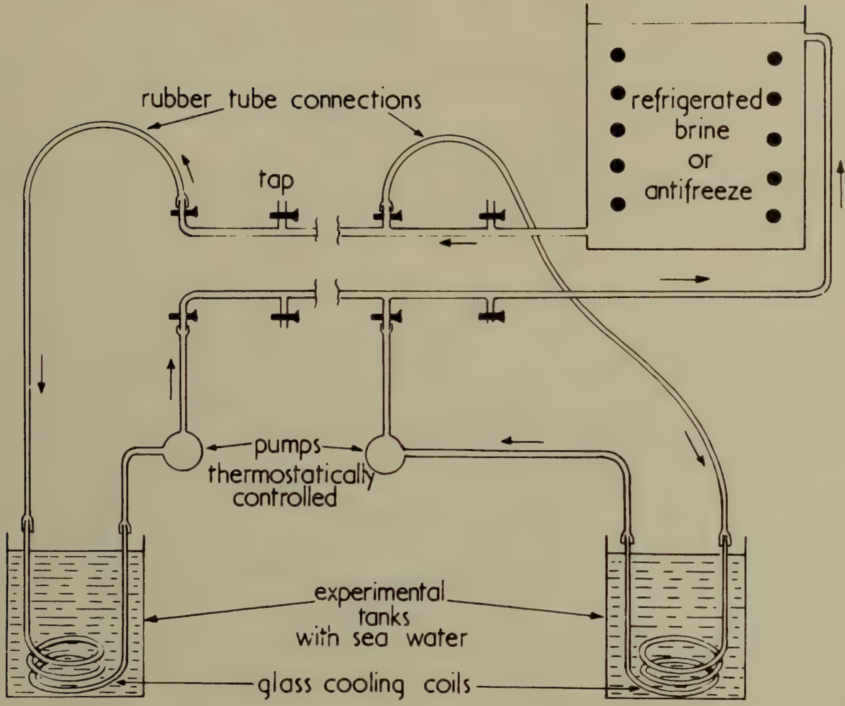
rubber tube connections
tap
refrigerated brine or antifreeze
pumps thermostatically controlled
experimental tanks with sea water
glass cooling coils

FIGURE 6.—Unit for coling a number of portable experimental tanks to varying controlled temperatures.

sure of the refrigerant. Check the rate of circulation of sea water over the refrigerator coils. Check heater functionally on temperature controlled tanks.

9. Clean up the aquarium and ancillary rooms (pumprooms etc.)

10. Remove any uneaten food material. Siphon off excreta and other debris from the tanks.

11. Make up sea water to the appropriate level in any isolated tanks.

12, Obtain supply of food material as required.

13. Make up daily diary.

Weekly maintenance

1. Take pH of the sea water and adjust if necessary.

2. Take salinity of the water, adjusting for evaporation where necessary.

3. Clean the glasses of the tanks.

4. Change over to the duplicate aerator motor.

5. Renew the sheets in the temperature-recorder units (two). Refill the pens with ink and check the accuracy of the recorder temperature. Check all thermostat controls functionally.

6. Check the level of the water in the reservoir tanks. Arrange for topping up if necessary. Ensure adequate reserves of sea water in tanks and carboys.

7. Change over to the alternate reservoir tank.

8. Clean out the aquarium and ancillary rooms.

9. Check all probes, including the action of the emergency probes, in both header tanks and sump.

10. Check functionally all circuit breakers, overload and other switches.

11. Check functionally all lighting and power points. Clean as necessary.

12. Clean and grease all sea-water pump motors. Check for corrosion. Clean off rust and repaint as necessary.

13. Examine outflow inspection chamber as a check on leakage and inspect pumprooms and all tank rooms for sea-water leakages.

Monthly maintenance

1. Drain, clean out, and reestablish each tank on a 6-month rotational basis.

2. Examine sea-water filters, renewing small individual filters as necessary.
3. Lift covers to ducts in north-block aquarium and hose out ducts. Clear rust from ironwork and touch up.
4. Clean and grease the refrigerator units (three) and blower motor.
5. Clean and grease aerator units (three).
6. Examine all machinery, lighting reflectors, conduit switches, etc. for corrosion. Clean off rust and touch up as necessary.
7. Remove Sunvic thermostats. Clean housings and control unit. Protect the bimetal strips by dipping in light oil.

Quarterly maintenance

1. Check air filters (three) for cleanliness; renew as necessary.

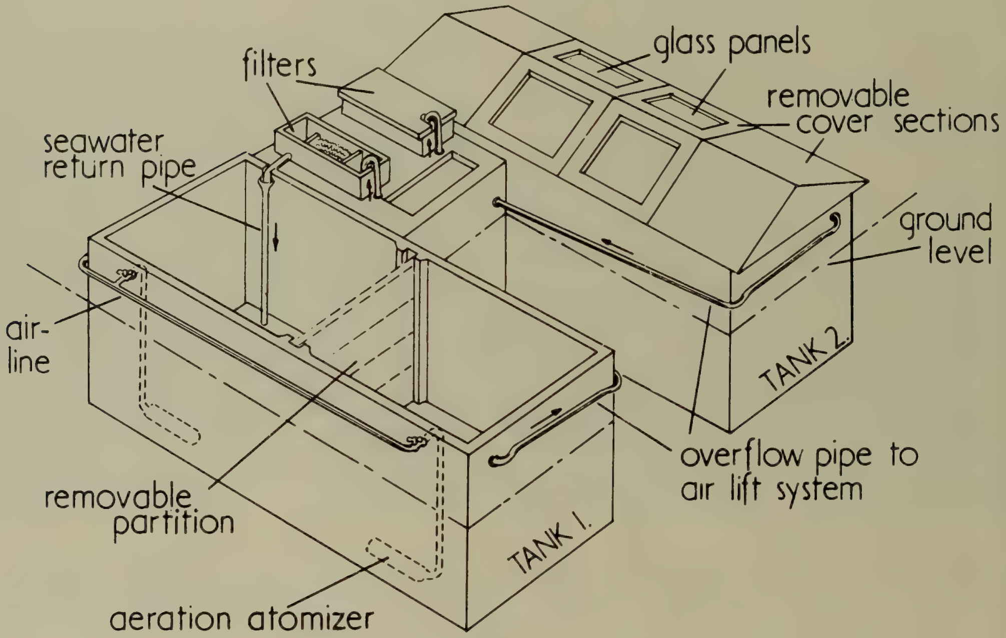

FIGURE 7.—Isolated experimental tanks. The covers to tank 1 and its associated filter unit, as well as the access cover to the inspection pit for the air lift, are removed. The sectional teak partition used to divide the tank is shown dotted in position, as also are the internal aeration pipes and atomizers.

A CLOSED CIRCULATING SEA-WATER SYSTEM

By M. S. Gordon and R. A. Boolootian

Department of Zoology, University of California, Los Angeles, Calif.

Abstract.—The closed circulating sea-water system of the Department of Zoology, University of California at Los Angeles, is described, and some parts are illustrated. Some important changes which would improve system operation are listed. These include changes in construction materials for large water tanks and sea-water return lines, and changes in the types of pumps.

The Life Sciences Building of the University of California at Los Angeles contains a large, closed circulating sea-water system which is used for both research and teaching purposes. This system has a capacity of about 25,000 gallons of sea water and has functioned very successfully since the fall of 1958. A wide variety of marine vertebrates and invertebrates have been maintained in the system for long periods.

STRUCTURE OF THE SYSTEM

The system extends through six floors of the Life Sciences Building. A basement room of the building contains two concrete storage tanks of about 9,500-gallon capacity each, two 1,300-gallon capacity sand gravity filters in redwood tanks, an 1,800-gallon redwood supply tank, associated hard-rubber and polyvinyl-chloride (PVC) unplasticized plastic piping, and several pumps (see figs. 1 and 2). From this basement room the sea water is pumped through a hard-rubber pipe to two 750-gallon capacity redwood gravity tanks on the fifth floor of the building (fig. 3). The water then circulates by gravity flow through hard-rubber and PVC plastic pipes to 3 aquarium rooms and 10 sea-water tables in various classrooms in different parts of the building. From these aquarium rooms and sea-water tables (fig. 4) the water returns to the storage tanks in the basement through Transite pipes.

The water is pumped from the main storage tank in use (one of the two storage tanks is used at a time, the other being kept as a reserve supply of sea water) into the two filter tanks by means of one of two small, neoprene-rubber-lined cast-iron centrifugal pumps (3-horsepower electric motors, Galigher Vacseal pumps, 1½-inch diameter outlet; fig. 2, *A*, *B*). From the filter tanks the water siphons into the central supply tank (fig. 1, *E*), from which it is pumped by a large supply pump (fig. 2, *C*) to the gravity feed tanks on the fifth floor. The head against which the main supply pumps operate is approximately 95 feet. There are two main pumps, but only one operates at a time, the second being kept in reserve. Both main pumps are powered by 10-horsepower electric motors and are cast-iron centrifugal pumps lined with high carbon content neoprene rubber (Galigher Vacseal, 2-inch diameter outlet). The pumps were originally designed for use as slurry pumps in mining operations.

All pipes and fittings in the supply part of the system are fabricated either of hard rubber or of unplasticized PVC. All

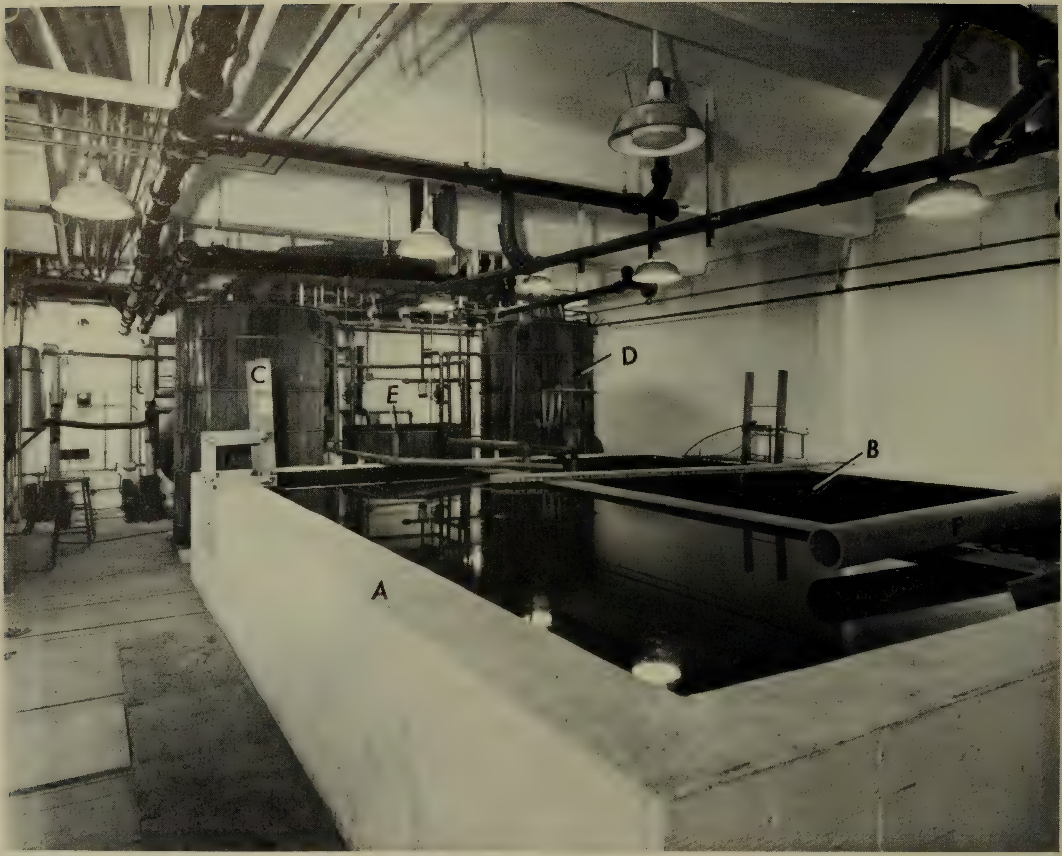

FIGURE 1.—Upper level of the basement pumproom. Sea-water system components include the two concrete main storage tanks (*A*, *B*), the two redwood gravity filter tanks (*C*, *D*), the redwood main supply tank (*E*), and one of two Transite pipes (*F*), leading from openings in the pavement outside the building, through which new sea water is added to the system. The Transite main return line from the building, suspended from the ceiling, crosses the center of the figure. Water returns to the storage tanks at the right-hand edge of the figure. Since this photograph was taken, the interiors of both storage tanks have been painted white with a nontoxic plastic paint. This facilitates their use as aquariums for larger animals.

large-diameter valves are of the diaphragm type, with neoprene-rubber gaskets. Water tables in aquarium rooms and classrooms are fitted with banks of hard-rubber stopcocks (fig. 4). They are also supplied with hard-rubber compressed-air lines. The water tables themselves are constructed of reinforced concrete.

Each of the three aquarium rooms has a large Pyrex glass countercurrent heat exchanger fitted into its sea-water supply line. Each of these heat exchangers is supplied with refrigerated fresh water from individual refrigeration units in adjacent parts of the building. Thermostats allow fixing of the temperature of the sea water leaving each heat exchanger.

Specific setups in the three aquarium rooms and on each of the classroom water tables are modified according to need. There is a general rule excluding all metal from the system. Accordingly, any aquariums with metal frames introduced into the system must be fitted with constant-level overflow siphons. In addition, rub-

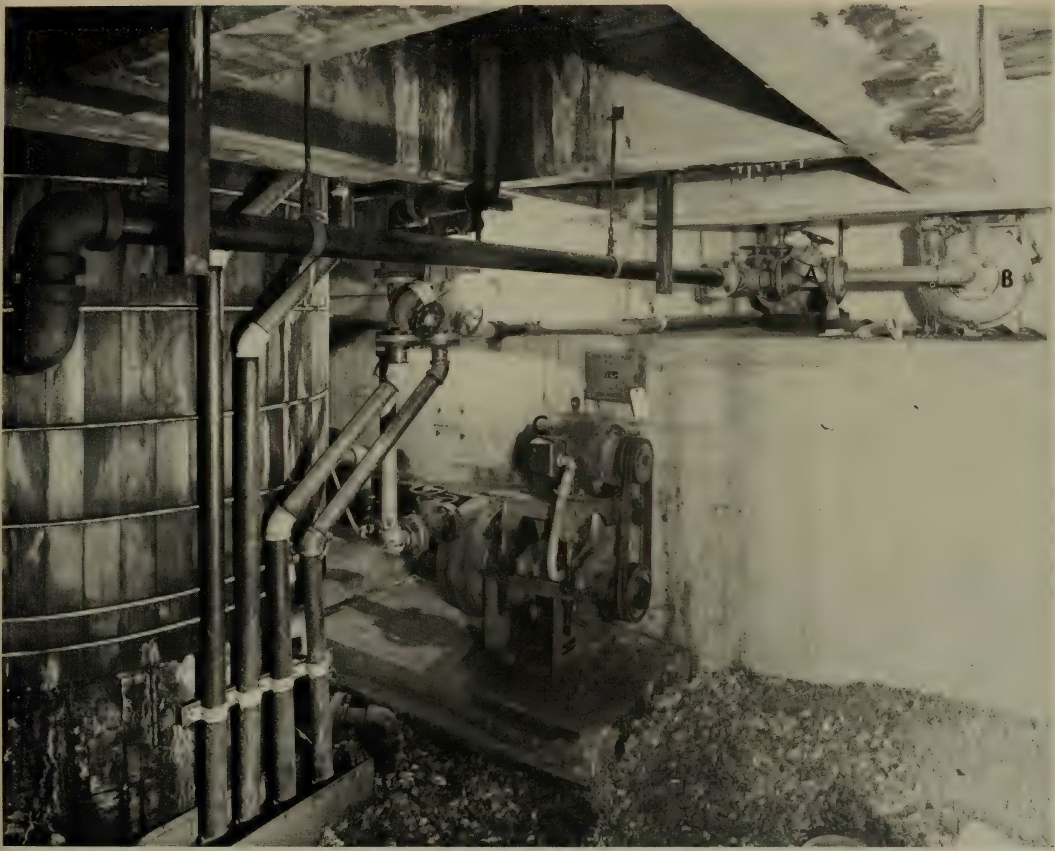

FIGURE 2.—Part of the lower level of the basement pumproom. Sea-water system components include the two small centrifugal pumps which move water from the main storage tanks to the filter tanks (*A*, *B*), one of the two centrifugal main supply pumps which pump water from the main supply tank (base visible on left) to the fifth floor of the building (*C*), and parts of four pipes. Two of these pipes are backflush drain lines for the filter tanks. The other two are bottom drain lines for the filter tanks. The bottom drain for the main supply tank is visible to the right of the lower ends of these four pipes.

ber hoses are avoided, Tygon tubing being used preferentially.

In operation of this system no attempt is made to maintain or control water quality over long periods. The most sensitive of the organisms kept in the system (octopuses, sea urchins, etc.) are used as indicators of deterioration. When signs of trouble appear (animals acting abnormally or dying) all water in the system is replaced with new sea water. The old sea water is drained from the storage tanks in the basement by pipes connecting with sewers. The new sea water is added via Transite pipes opening through the sidewalk outside the building (fig. 1, *F*). We charter stainless-steel milk-tank trucks to transport sea water to the system from Marineland of the Pacific, Palos Verdes, Calif. Sea water replacement is necessary only about once every 2 to 3 months.

One of the major reasons for the relatively long period of maintenance of water quality despite a heavy load of animals is the efficiency of operation of the sand filters in the basement. These fil-

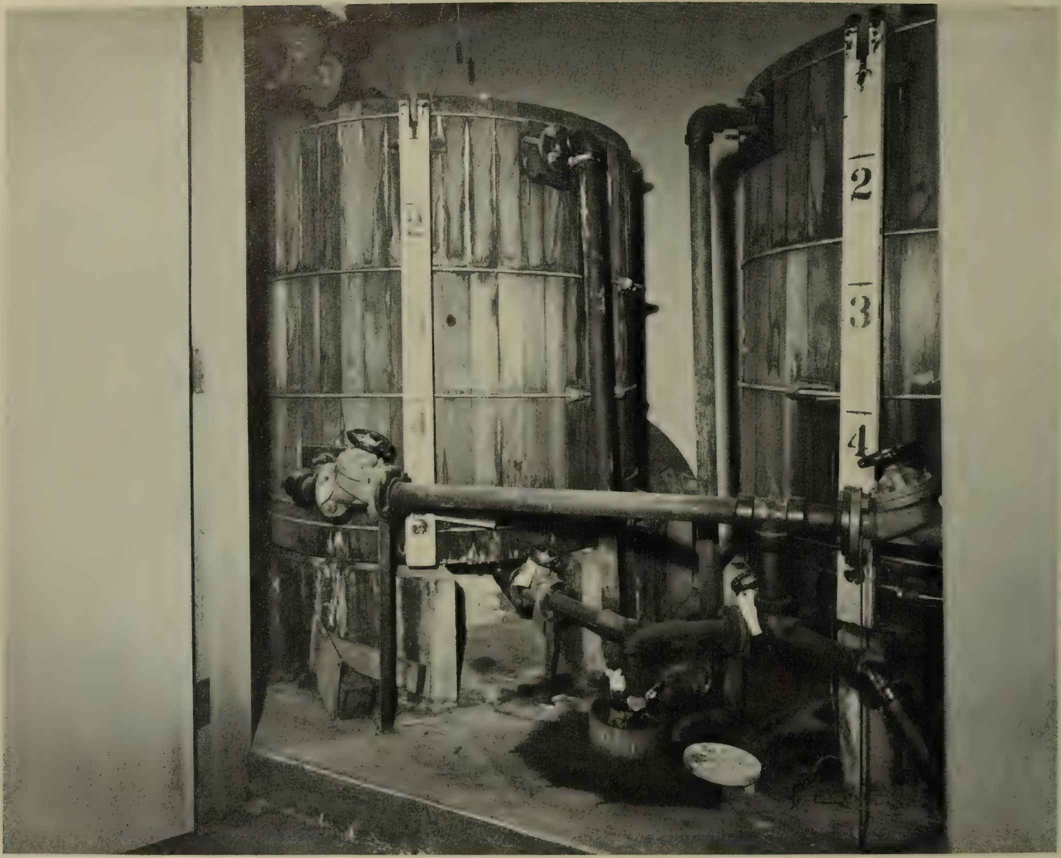

Figure 3.—The two redwood gravity-feed tanks on the fifth floor.

ters maintain a considerable bacterial population which seems to remove much toxic material from the water. The only maintenance necessary on these filters is occasional (about every 4 or 5 months) back-flushing of the filters with fresh water. Each filter tank is fitted with suitable fresh-water and compressed-air lines, and back-flush drain lines, for carrying out this operation with minimum loss of sea water.

The system is equipped with an alarm system which immediately notifies the University maintenance department of any interruption in operations. Maintenance men are available on short notice at all times.

RECOMMENDATIONS

As it is, the system functions very well. However, if redesign of the entire system were practical several things would be done differently.

First, we would not use centrifugal pumps as motive power. Centrifugal pumps are relatively inefficient in terms of the amount of power needed to pump a given volume of water a given distance. This is especially true for the rubber-lined pumps we use in order to avoid metallic contamination. Recent developments in pump design have made available several other more satisfactory types of pumps for continuous surge-free operation against large pressure heads. Surge-free

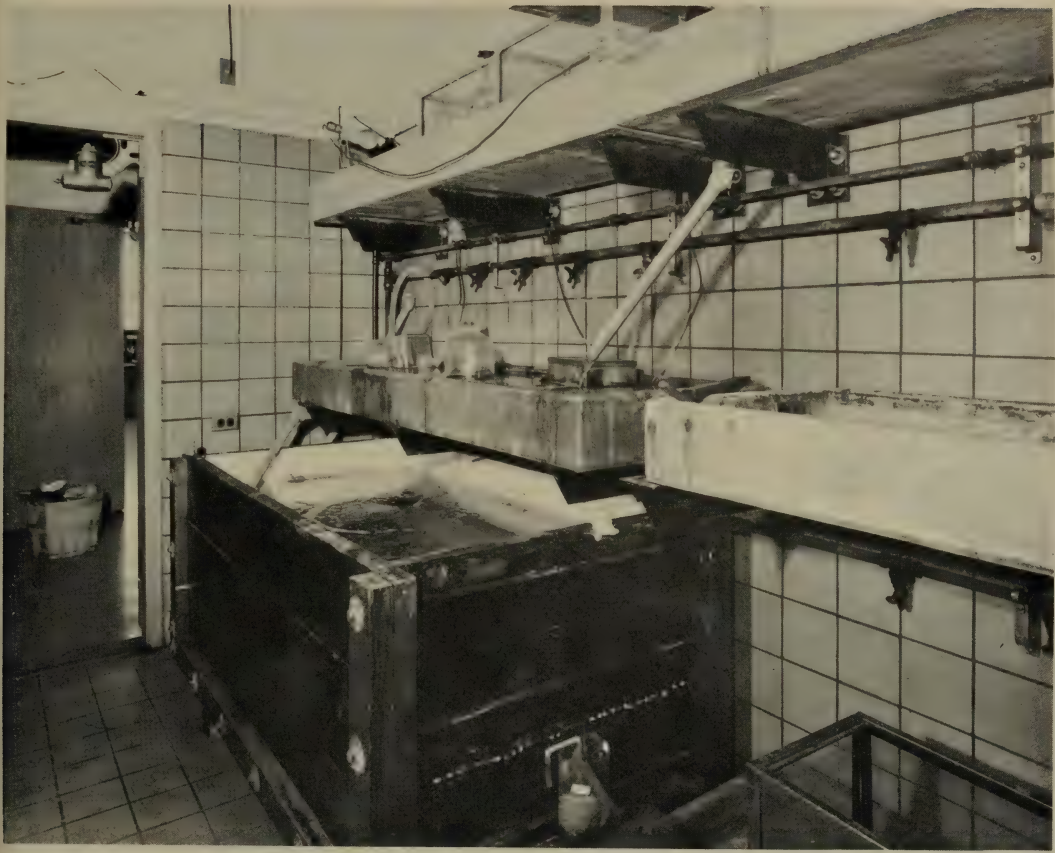

FIGURE 4.—One of the three aquarium rooms showing several reinforced-concrete water tables (approximate dimensions 72 by 22 by 4 inches) and a large fiberglass-lined redwood tank (250-gallon capacity). Note banks of stopcocks on wall, also compressed-air line with multiple outlets.

operation is especially important because hard-rubber and PVC plastic are both brittle and break easily when distorted.

Among the alternative pump designs which seem attractive are gear pumps with Teflon plastic gears and pump-case linings and Archimedes-screw pumps with plastic-coated screws and case lining.

Second, we would not use redwood as a construction material for any tank in the system. Redwood continues to leak colored materials into the water over long periods—this is still happening in our system after over 3 years of continuous operation. This colored material appears not to be toxic or in any way pharmacologically active, but it mars appearance of the sea water and hence interferes with observations. Fiberglass-reinforced plastic or wooden tanks would seem to be the solution.

Third, we would not use Transite as material for return lines. Transite piping is permeable to water and will, therefore, accumulate salt deposits on its outer surfaces if used to transport sea water for long distances through air spaces. These salt deposits do not seem to weaken the transite itself, but can be the cause of important building deterioration if atmospheric water condensation occurs which then results in drip of salt water. The solution is the replacement of all transite with unplasticized PVC pipe.

ACKNOWLEDGMENTS

Continued successful operation and maintenance of this system would not be possible without the able cooperation of the UCLA Department of Buildings and Grounds. Assistant Chief Engineer John Little was largely responsible for the expert and expeditious solution of many problems which arose during the difficult early "shake-down" stages of the operation.

ADDENDUM

In the time since this paper was submitted, new developments have occurred in pump technology which have revised our opinions concerning centrifugal pumps. Several very efficient, reliable, nontoxic centrifugal pumps adequate for the needs of the UCLA system have appeared on the market. The two main supply pumps and one of the filter pumps in our system have been replaced with these. The casings and the impellers of these pumps are made of one of several types of epoxy resin. One of these pumps (a Series P Durcopump, manufactured by Duriron Co., Inc., Dayton, Ohio, powered by a 3-horsepower motor) has now (December 1963) been used continuously as the main supply pump for the system for over 1½ years. There has been no down time, and the pump has required only routine maintenance.

A MEDIUM-SIZED SEA-WATER SYSTEM FOR THE LABORATORY

By J. E. McInerney and W. S. Hoar

Department of Zoology, University of British Columbia, Vancouver, B.C.

Abstract.—A simple and efficient salt-water system which may be easily constructed from readily available materials is described. Various ways of solving the problems of contamination, aeration, removal of waste products, and temperature maintenance are considered.

The principal problems in maintaining a closed salt-water system in the laboratory are as follows:

1. Avoidance of contamination.
2. Maintenance of adequate oxygen levels.
3. Removal of waste products.
4. Maintenance of temperatures.

The sea-water system described here provides a reasonably simple arrangement of a size intermediate between the typical home aquarium and the large public aquarium. It has operated satisfactorily in our laboratory for many years and has been found useful in maintaining teaching and limited research material. A schematic representation is given in figure 1. Solutions to the problems listed above are achieved in the following ways.

CONTAMINATION

All parts are constructed of wood (filter tanks and aquariums) or rubber or plastic (pump, pipes, and connections). Where it is necessary or more convenient to use metal, these parts are coated with nontoxic rubber- or oil-base paints. If it is impractical to paint exposed metal, then a suitable type of noncorrosive stainless steel is necessary. For example, the pump shaft is frequently constructed of this material.

OXYGEN

Adequate oxygen levels can be maintained in several ways. The most efficient system involves a supply of compressed air delivered through plastic tubing to air stones with plastic rather than metal stems. As an auxiliary source of aeration, the water returning from the elevated filter can be sprayed back into the aquariums. Similarly the water supply to the filter can be sprayed onto the filter bed by taking advantage of the pressure supplied by the pump. The amount of aeration required is a matter of experimentation depending mainly on the temperature of the water and the number of animals.

WASTE MATERIAL

A useful and simple filter arrangement is shown in figure 1. Water is removed from a point as far as possible from the clean-water inlet. It is pumped by a self-priming plastic pump to the elevated filter tank. An intermittent rather than continuous pumping action is achieved by using a plastic float (in our case, a toilet-tank float) in conjunction with a mercury switch (similar to those found in furnace thermostats). Details of this system are shown in figure 2.

The filter bed may be constructed of any

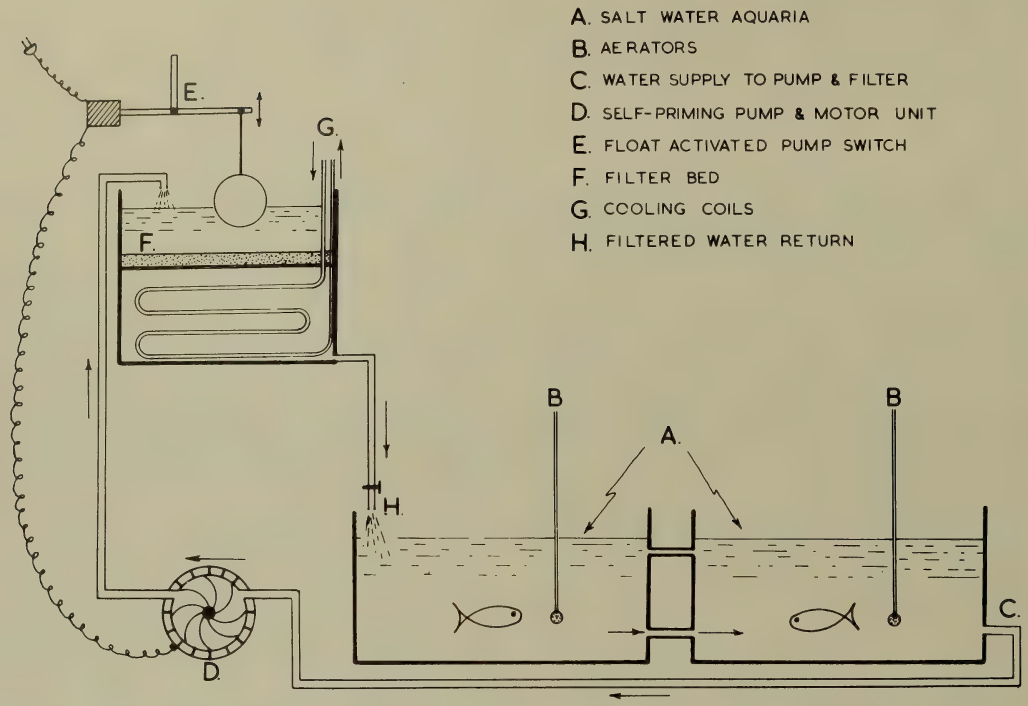

FIGURE 1.—Salt-water system: *A*, salt-water aquariums; *B*, aerators; *C*, water supply to pump and filter; *D*, self-priming pump and motor unit; *E*, float-activated pump switch; *F*, filter bed; *G*, cooling coils; *H*, filtered-water return.

suitable nontoxic material. Two types are illustrated in figure 2. Successive layers of finer and finer gravel beginning with the coarsest gravel or rock at the bottom provides the least expensive method of filtration (fig. 2, *1*). Cleaning this filter bed involves running fresh water into the bottom and up through the sand and gravel to the top of the tank (fig. 2, *3*) to waste through an overflow outlet (fig. 2, *4*).

A simpler disposable filter is made from two thicknesses of industrial fiberglass battens (fig. 2, *2*) mounted on an appropriate frame. The frequency with which the fiberglass must be washed or changed will depend mainly on the number of animals in relation to the surface area of the filter bed.

TEMPERATURE

With a closed salt-water system there must usually be some means of keeping the water sufficiently cool. The simplest and least unsightly method is illustrated in figure 2. A supply of cold tap water is circulated through plastic or, for more efficient heat transfer, stainless-steel coils mounted below the fiberglass filter bed (fig. 2, *5*). With a graded-rock filter the coil may be included directly in the filtering material. More precise temperature control may be achieved by using either a complete refrigeration system or by operating a thermostatically controlled heater in opposition to a continuous source of cooling such as the cold-water coils suggested above.

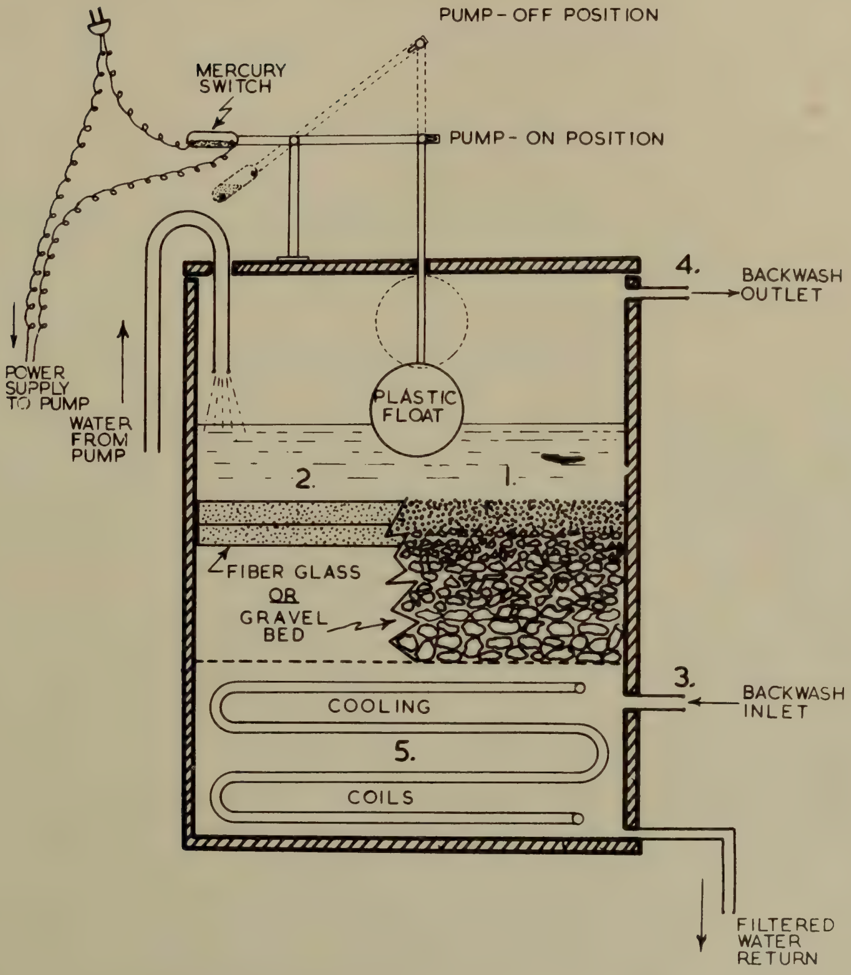

Figure 2.—Filtering and cooling details.

Alternatively, the cooling coils (glass tubing is satisfactory) may be included in the aquariums or the aquariums may be surrounded by a bath of running water.

The system illustrated here may, if desired, be very simply constructed of materials found in most laboratories. A wooden barrel will serve as a reservoir, and ordinary glass aquariums may be set in series with water siphoned from one to another. If the pump is located below the aquariums, it need not be self-priming. Our filter tanks are about one-third the capacity of the associated aquariums, but this is largely a matter of convenience since the rate of filtering can be adjusted.

MINIATURE CIRCULATING SYSTEMS FOR SMALL LABORATORY AQUARIUMS

By C. M. Breder, Jr.

The American Museum of Natural History, New York, N.Y.

Abstract.—Methods are given for constructing both an open system, wherein the sea water runs to waste, and a closed system, wherein the water is recirculated. Easily available materials are used, and all devices, such as a constant-level siphon and an automatic shutoff, are simple to assemble. Each of the systems is designed so as to require a minimum of maintenance. Equipment necessary for the control of sea water is discussed.

Because of the requirements of certain experiments it became necessary to establish various small, but fully controllable circulating systems in small aquariums. These have included both open and closed fresh-water systems and closed salt-water systems. As the designs eventually worked out have proved to be entirely satisfactory, and as many colleagues have inquired about these systems, with a view to building similar ones for their own purposes, the details of construction and operation are explained here.

Primarily these systems are the outgrowth of work of earlier years at the old New York Aquarium, where much larger, but similar, equipment formed the basis of operations. This equipment itself had been developed from schemes used by older institutions of similar kind. Naturally, many persons had a hand in developing the arrangements and devices employed at the New York Aquarium. For these reasons the origins of the devices were not always clear, but those chiefly interested and responsible for them at the Aquarium were C. W. Coates and the late C. H. Townsend, and H. Knowles. Townsend (1928) and Breder and Howley (1931) reported on some of these features. It has been found that by suitable modification of the principles of the larger devices it is possible to develop very useful miniature equipment. Such need, of course, applies only to laboratories which are not connected with large public aquariums and which consequently lack the utilities usually to be found only in such places. These devices have been worked out in connection with experimental work carried on in the laboratories of the Department of Fishes and Aquatic Biology of the American Museum of Natural History, which has been supported in part by the National Science Foundation.

OPEN SYSTEMS

An "open system," as the term is used here, is one in which the water is used once and is not recirculated; there is only a supply line and a drain line. This calls for little comment in present connections except where a very small, well-regulated flow is required. Such apparatus may be

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arranged to provide as little as a specified number of drops a minute, and will maintain a surprising accuracy if properly designed.

The overflow provided for this system is a constant-level siphon. If such a siphon is made by a glass blower, it will be expensive, subject to breakage, and not readily cleaned. A siphon can be made quickly and cheaply of some straight glass tubing, a tee, some flexible rubber or plastic tubing, and two small strips of wood or plastic. No dimensions are given, as these will vary with the individual needs, although figure 1 is drawn to scale. The two strips of plastic are identical; each has two holes drilled in it to fit the glass tubing snugly. The parts are assembled to make a constant-level siphon attached to the lip of an aquarium as shown in figure 2. The lower piece of plastic may be fastened to the aquarium by small clamps or may be cemented to it. The open, upper end of the tee vents the siphon. If a cap or plug is placed on this it immediately becomes a simple siphon and will drain the aquarium to the level of its inlet tube. This is sometimes found to be an added handy feature. The level of the water in the aquarium will be that at which the overflow water spills out through the horizontal leg of the tee. Adjustments of this to a fine point may be made by raising or lowering the tee through the hole in the plastic support, or this whole external assembly may be moved up or down by altering the position of the straight length of tubing which passes through the hole in the other piece of plastic connecting it with the tube inside the aquarium. By arranging the outside part of the siphon to lie along the aquarium wall, as shown, the danger of its being in the way of operations is reduced.

Cleaning presents no problem with this type of siphon. If something does nevertheless block the siphon tube from the aquarium, it almost always can be cleared by blowing into the open end of the tee and restarting the siphon by drawing on this same open end of the tee while the outlet tube is held shut. It is possible and sometimes more convenient to attach the outer portion of the constant-level

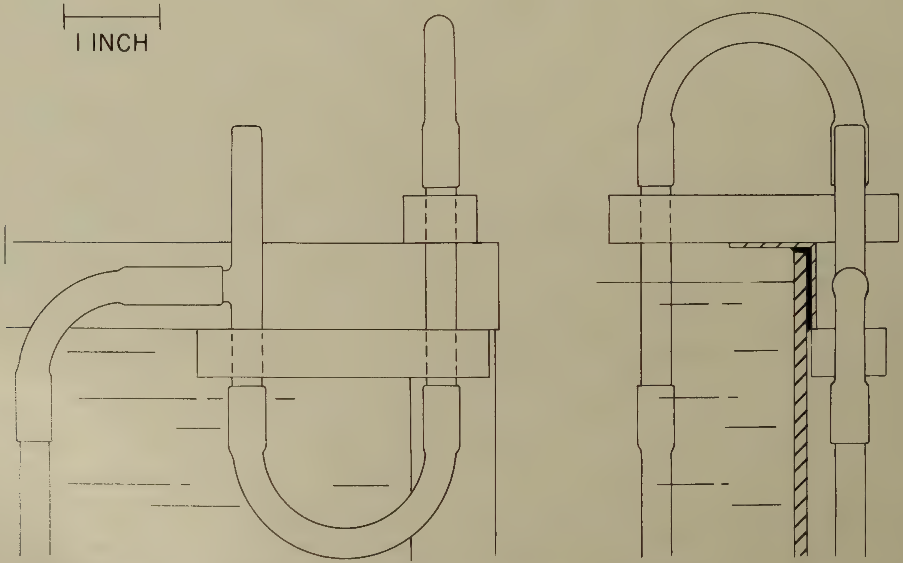

Figure 1.—Side and end view of constant-level siphon made up of sandard parts.

siphon to a small board which is affixed to a pivot at its upper end so that it is free to rotate on the stationary part attached to the aquarium frame. A small handle pointing upward from there makes its adjustment simple and marks on the latter in reference to some stationary part make return to a former rate of flow exactly possible. The action is simply that by rotating the part of the siphon so that the horizontal part of the tee raises or lowers, the level in the aquarium follows accordingly. This in turn affects the float valve, which is described below. The rate of flow will increase if the siphon outlet is lowered and decrease if it is raised. This is useful where the exact level of water is not of any importance but where it is desired to vary the amount of water flowing through the aquarium by specific amounts and where it is necessary to repeat such changes in flow at will.

If the water supply has considerable pressure, such as is ordinarily encountered in city water systems, or approaches it, a pressure-reducing valve which may be regulated should be employed. This can bring the pressure down to a value which will not burst or otherwise destroy the light equipment to be employed. This valve placed someplace in the supply line should be set so as to deliver little more than the maximum amount of water which will be required of it. Another way to accomplish the same purpose is to permit the supply water to run into a small reservoir of no more than sufficient height to provide enough head of pressure. Into this reservoir the supply water is allowed to run continually, of a little more volume than the aquariums will ever need. This is necessary to maintain a constant head in the reservoir. A small excess will overflow and go to waste by this method. It is

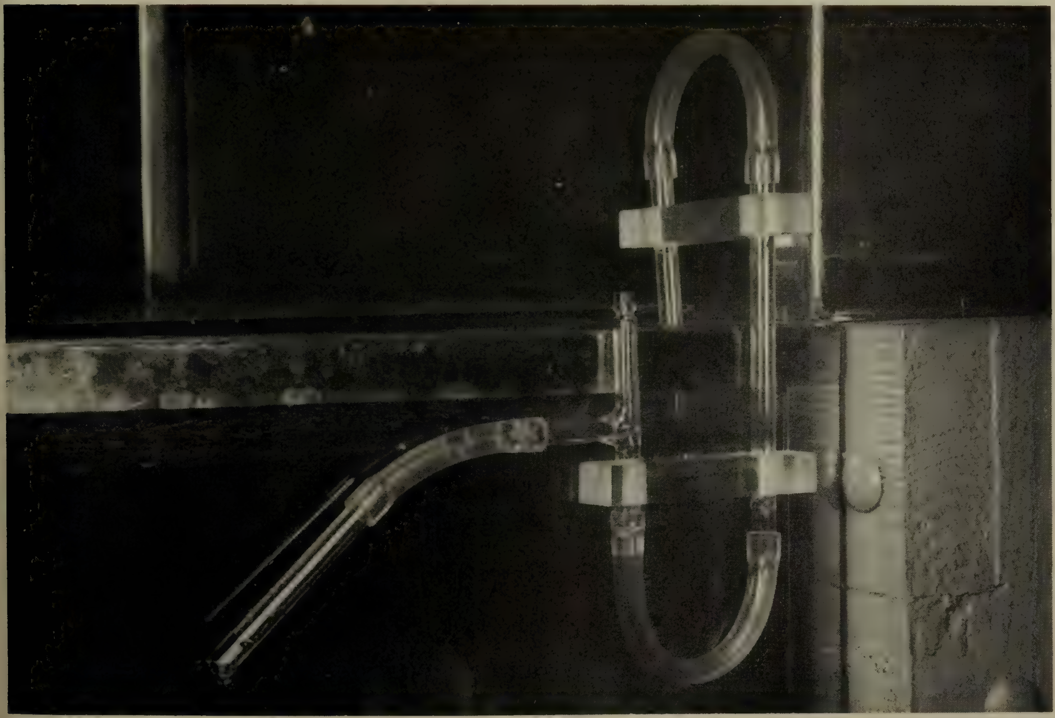

FIGURE 2.—Constant-level siphon in operation.

economical only where water saving has no significance.

The water flowing into this aquarium is controlled by a float valve constructed of a glass stopcock, a suitable-sized chemical flask, and some small parts of either wood or plastic. These are assembled as shown in figure 3 and are held together by iron screws and two pieces of strip steel. This metal is mentioned because of the danger of toxic salts forming if brass were used, since such corrosion might fall into the aquarium. A 1-hole cork is bolted to a piece of lucite and then inserted into the mouth of the flask as shown. A dowel or plastic rod is inserted in the other hole in the plastic piece and secured. A similar piece of plastic is movably secured on this rod and on a similar one at right angles to it, extending from the valve. This is so arranged that the center of the flask comes to rest directly below the horizontal rod extending from the valve. This is best seen in the plan view of the device. By loosening the two setscrews in the upper plastic piece the flask may be moved vertically on the one and horizontally on the other. Figure 4 shows one arrangement of this device.

The extending glass tubes which are an integral part of the stopcock are inserted through two snug holes in the wood or plastic endpieces of the valve, and these are held in position by the two steel strips which are held in place by four wood screws. In the center of one of these steel pieces a hole is tapped into which is screwed a setscrew with a pointed end (about 60°) and a locknut as shown. The stem handle of the stopcock is imbedded in a piece of wood cut about as shown. For this purpose a suitable space is hollowed out in the block into which the stem is inserted, the space around being filled with plastic wood or a similar product. The face of the block and the stopcock stem must be at right angles. On the outer face of the block a small steel strip is affixed with a small drill-tip impression at its center on the axis of the stem. Into this the pointed setscrew fits as shown. This is adjusted so that the valve works freely without being too tight or leaking. This prevents the glass stopcock from working loose and leaking after long-continued operation. The diagonal dotted line on the block indicates the position of the hole through the plug as well as that of the handle on the stem. It is shown in a position just fully closed. It is obvious that with a fall in the water level the valve will open proportionally to the change in water level and shut itself off as the water level rises.

The interaction between the constant-level siphon and this valve is indicated in figure 5. It is clear that danger from flooding could come only from some damage to the equipment. If, for instance, something clogs the overflow in any way, the float valve shuts itself off when it has reached the predetermined point for which it has been set.

As an extreme point of precaution a safety alarm or shutoff could be built as an entirely separate system. Such a device, which has never failed so far as the writer's experience goes, consisted of an old pair of contacts such as are to be found on relays, to one member of which was fastened a shell vial. This hung over the water in such a manner that when the water rose over a specified place it lifted the vial and pushed the two contacts together. It operated on two dry cells to ring a doorbell but could be used with a relay to switch on house current to operate any suitable device. This could be a normally open solenoid valve placed in the supply line. Such extreme caution would only be warranted where a little flooding would be disastrous.

It is obvious that this float valve could be used under certain experimental pro-

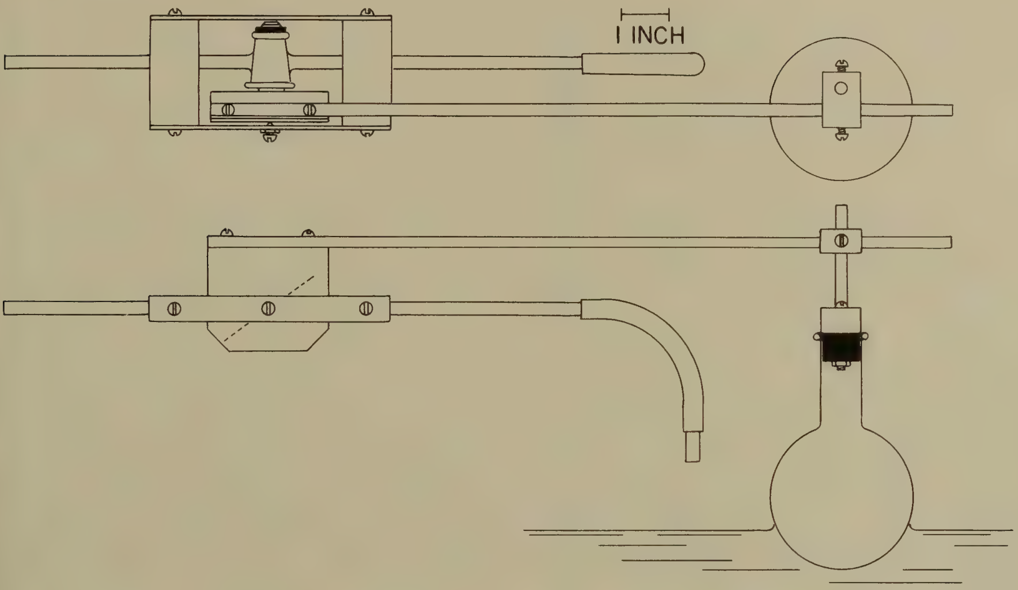

FIGURE 3.—Top and side view of float valve for control of inflow of water and additives.

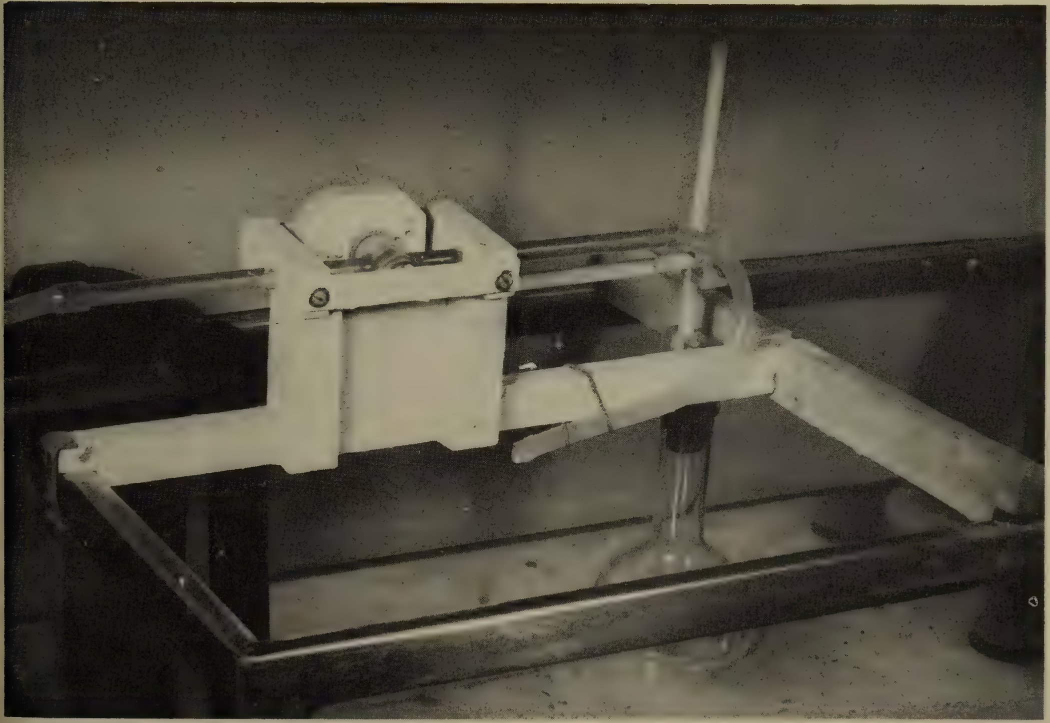

FIGURE 4.—Float valve for control of inflow. This is the arrangement in the closed salt-water system; the placement is for convenience, but usually valves are placed close to one end.

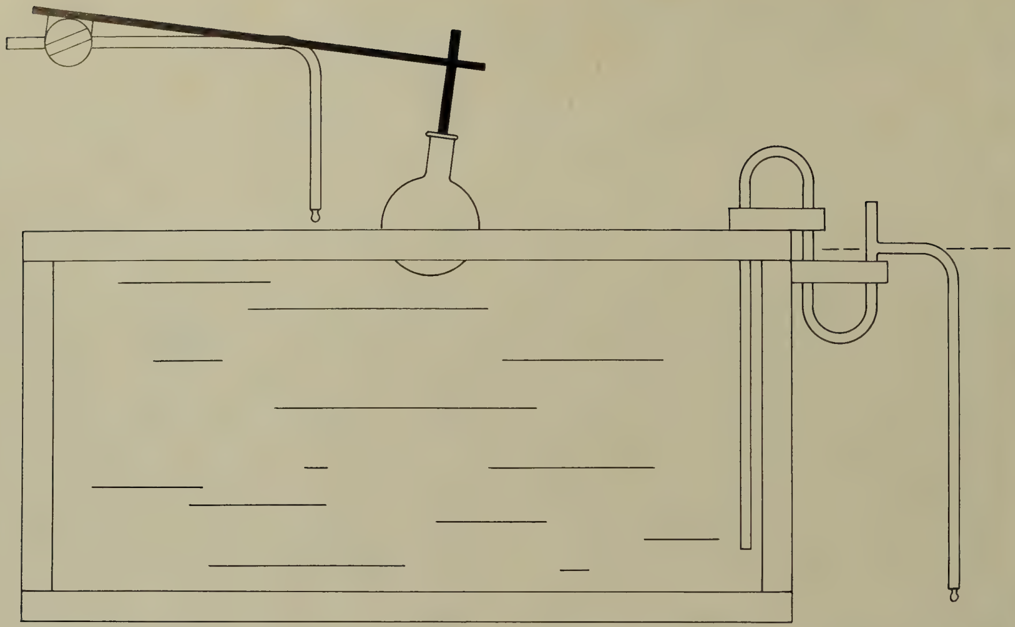

FIGURE 5.—Diagram of interaction between float valve and constant-level siphon. Dashed line represents level of water in aquarium and siphon arm.

cedures to add chemicals to an aquarium at a prescribed rate by inactivating the float and fixing the rate of flow by hand. It also could be used to bring the concentration of some chemical to a fixed limit and then hold it at that point in flowing-water aquariums. The water supply would operate as above described and a second float valve regulated to add much less chemical than the water flow would move with it and act as a follower to the other if there was any fluctuation in the flow of water, thus holding the additive in proportion to the change of water. Also a single float could be arranged to operate the two valves in proportion to the setting of each.

While the designs of these float valves have varied from time to time, all have embodied the same principles as herein described. The first and somewhat primitive one has, at this writing, been in service continually for more than 4 years and is still entirely satisfactory and dependable.

CLOSED SYSTEMS

The term "closed systems" refers to circulating systems in which the water is returned to the aquariums after filtration or other treatment and none is allowed to run to waste during normal operations. Some such system is mandatory for the maintenance of marine forms remote from a ready supply of sea water, and often convenient or necessary for various experimental procedures involving fresh-water aquariums. This is especially true of cases where it is necessary to maintain close control of some feature such as temperature, chemical quantities, and the like. By use of such means it is possible to maintain a series of aquariums with absolutely identical water conditions, as the water in all is part of a common body. Consequently no matter what transpires in one aquarium there is no opportunity for the water of that one to depart from the characteristics of the rest since it is moving freely from one aquarium to the other and is being continually and effectively mixed.

An especially useful arrangement for some purposes is one in which the flow between aquariums may be continuously varied from maximum in one direction through zero flow to maximum flow in the opposite direction. This may be readily accomplished by the adjustment of four valves while the pump runs continuously in one direction at constant speed. The details of the arrangement of these valves are shown diagrammatically in figure 6.

Figure 7 is a photograph of such a device. In operation the action is as follows. With valves *A2* and *B1* closed and the others open, the flow is out through pipe *A* and returns through pipe *B*, as indicated by the arrows, at maximum flow. If these valves are reversed so that *A1* and *B2* are closed and the others open, the flow through pipes *A* and *B* is reversed, although the flow through the pump remains as indicated by the arrow on it. To pass uniformly from the first position, as shown in figure 6, through a state of no flow to the reverse, either valve *A2* or valve *B1* can be gradually opened. This reduces the speed of water movement because of "back leakage." After one of them has been opened fully, the opening of the other can further retard flow. When it, too, has been fully opened, that is, with all valves fully open, there should be no flow through pipes *A* and *B*, as there is as much pump pressure in one branch of both *A* and *B* pipes as in the other. Then by beginning to close either valve *A1* or valve *B2* the flow begins to move in the opposite direction. When these two are fully

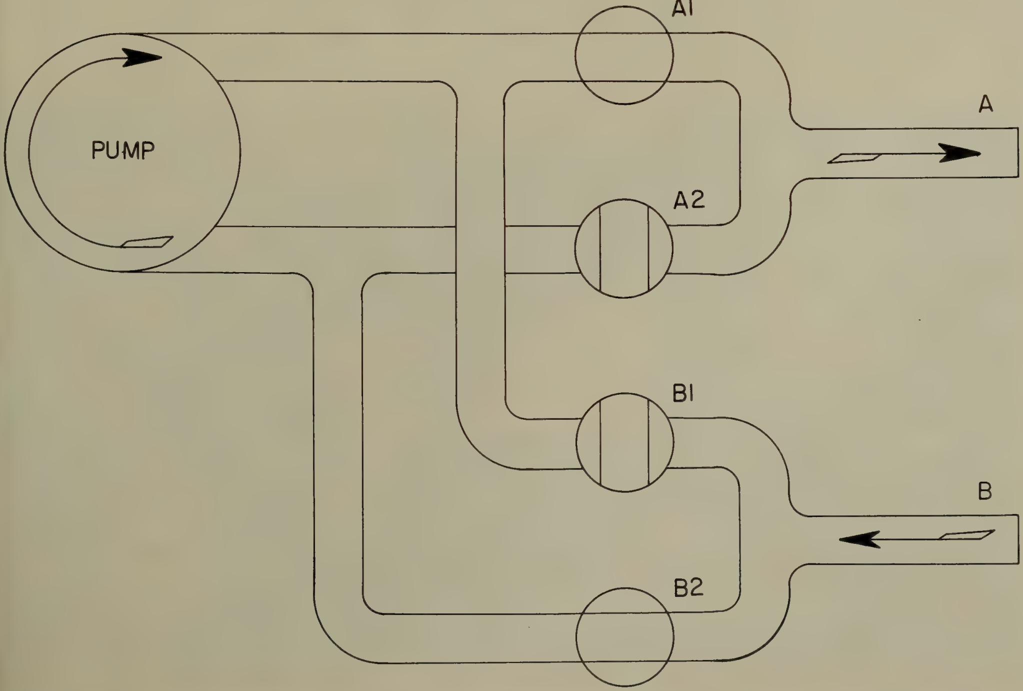

Figure 6.—Diagram of piping and valves for continuously variable flow from maximum in one direction through zero flow to maximum in the opposite direction.

closed, the maximum flow in the opposite direction has been reached.

If three valves are arranged on either line *A* or line *B*, or such a set on both, various water-treatment devices may be placed in the series, such as a heating or cooling device, in which case the water may best be passed through a glass coil for heat exchange. This arrangement could equally well be used for any kind of decontamination which might be required or for the introduction of specified materials being mixed with the passing water. Unlike most aquarium plumbing, in this case the water must pass from one aquarium to another, so that as many as may be needed can be placed in series.

A diagram of a more usual arrangement for a closed circulating system is shown in figure 8. This is the form which is perhaps most useful for general laboratory purposes. Here each aquarium is supplied and drained directly from a common supply and return. It is advisable to use a pump of somewhat greater capacity than needed for the purpose. With this means the excess water can be returned to the reservoir without passing through the aquariums, which greatly assists in the efficient application of whatever water treatment is being given and insures adequate pressure for the system. Simple filters may be made by wedging a piece of glass in a small aquarium and filling the intake side with suitable filter material, while the reservoir may be used for whatever chemical or other treatment is to be applied. Two may be provided, as shown in the figure, and used alternately or together. A constant-level siphon takes the water to the first reservoir aquarium. Only one siphon is shown, which may be switched to the other aquarium for cleaning purposes.

The pipes and fittings used in this system are standard hard rubber. Connections between aquariums at the same level may be made by "jumpers" which are preferably of hard rubber. Their use is indicated in the lower-level aquariums

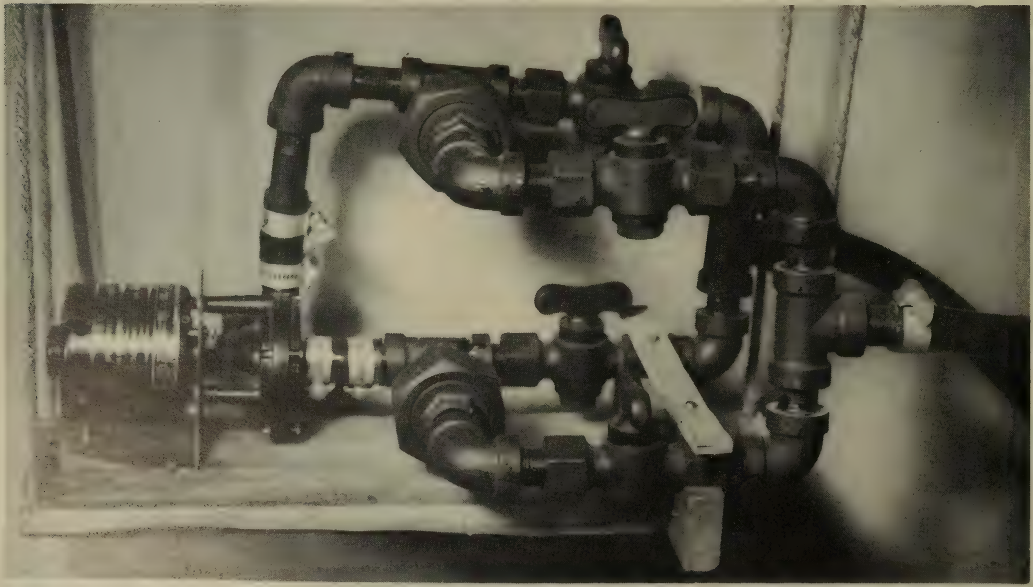

FIGURE 7.—Pump provided with reversible-flow device. The plastic pump here shown is powered by a 1/80-hp. motor and is rated to deliver 3.7 gallons per minute at zero head.

shown in figure 8. These have been found to be fully satisfactory and in several years' operation have not clogged nor have they become airbound. They are, however, not suitable for the overflow lines of the upper series of aquariums. Here constant-level siphons may be used as shown in figure 2, or, preferably, a hole may be drilled in the slate bottom of each aquarium and a 1-hole rubber stopper holding a glass tube inserted. Still better is the installation of a hard-rubber standpipe locked in place with fittings. The drilling of slate is not easily accomplished and there is considerable danger of cracking or otherwise damaging the aquarium. The manufacturers will supply aquariums with such holes drilled on order.

Since the supply to the aquariums of this system is preferably from the top, as indicated, the drain line as above described may be made to draw water from the bottom of an aquarium by the following simple means. A tube of glass, or other material, of larger diameter than the drain tube and as long as the depth of water in the aquarium, is placed over it, reaching nearly to the bottom of the aquarium. Since the larger tube extends above the surface, water leaving the aquarium must enter the annular space between the two tubes and pass up between them to spill into the open upper end of the inner tube. In addition to giving the aquarium a better circulation, much detritus is drawn up through this arrangement and delivered automatically to the filters. The outer tube, if of glass, may be positioned by slipping a short piece of plastic tubing on its lower end and cutting various openings or notches in the plastic. The area of these passages should of course be at least equal to the cross-section area of the inner tube. The annular space between the inner and outer tubes should also have this much area, at least. On the other hand, it is best not to make the annular space much larger than needed because

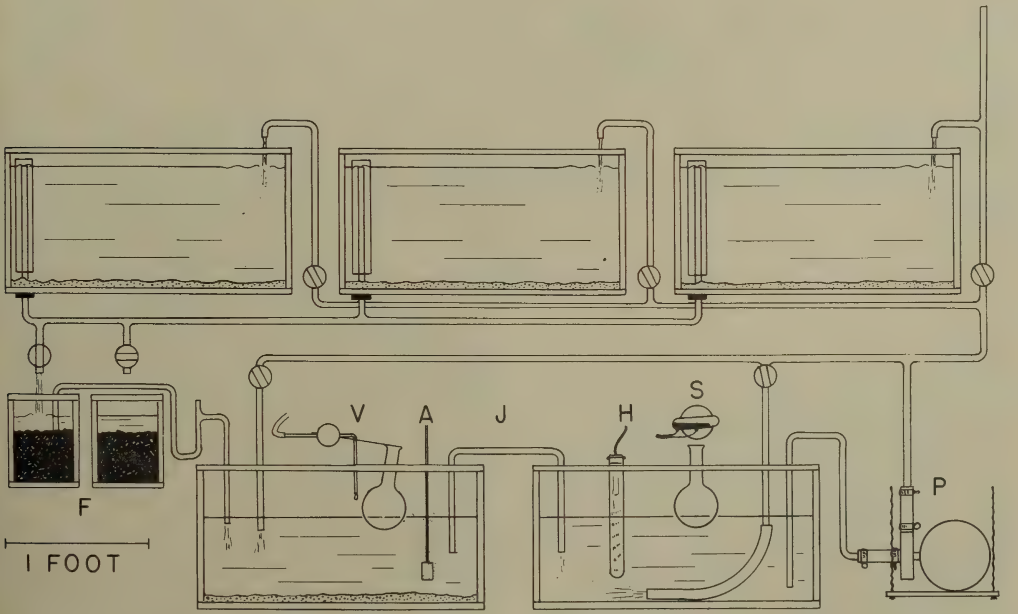

Figure 8.—Arrangement of a closed circulating system suitable for small marine aquariums: *A*, aeration outlet; *F*, filters; *H*, heater and thermostat; *J*, jumper; *P*, pump on hanging support; *S*, safety cut-off switch; *V*, float valve. The distilled-water supply carboy is not shown.

this will cause the water flowing through it to move with less speed. The value of this arrangement as a detritus remover is thus lessened, for the slower-flowing water will not lift as heavy particles as will the faster.

As such a system is usually intended to be operated continuously for long periods without attention, a safety feature may be built in which would shut down the pump if the water in the reservoir should rise too high or fall too low. The one in current use, shown in figure 9, was improvised from the tube of a mercury switch. This was mounted on a rotatable glass shaft running through a support of plastic. It was actuated by a chemical-flask float by means of a thread over a small drum so that the motion of the float was transmitted to the pump switch. Any unusual change in the water level, either positive or negative, would indicate some radical failure at some point in the system. Since the aquariums which held the fish were drained by an overflow, they would continue to hold their water level so that stopping the pump would insure the retention of water there. Even if one of the aquariums leaked and lost its contents the others would not suffer by draining through the system to it because of this protective device.

To prevent normal evaporation from stopping the pump, a supply was provided

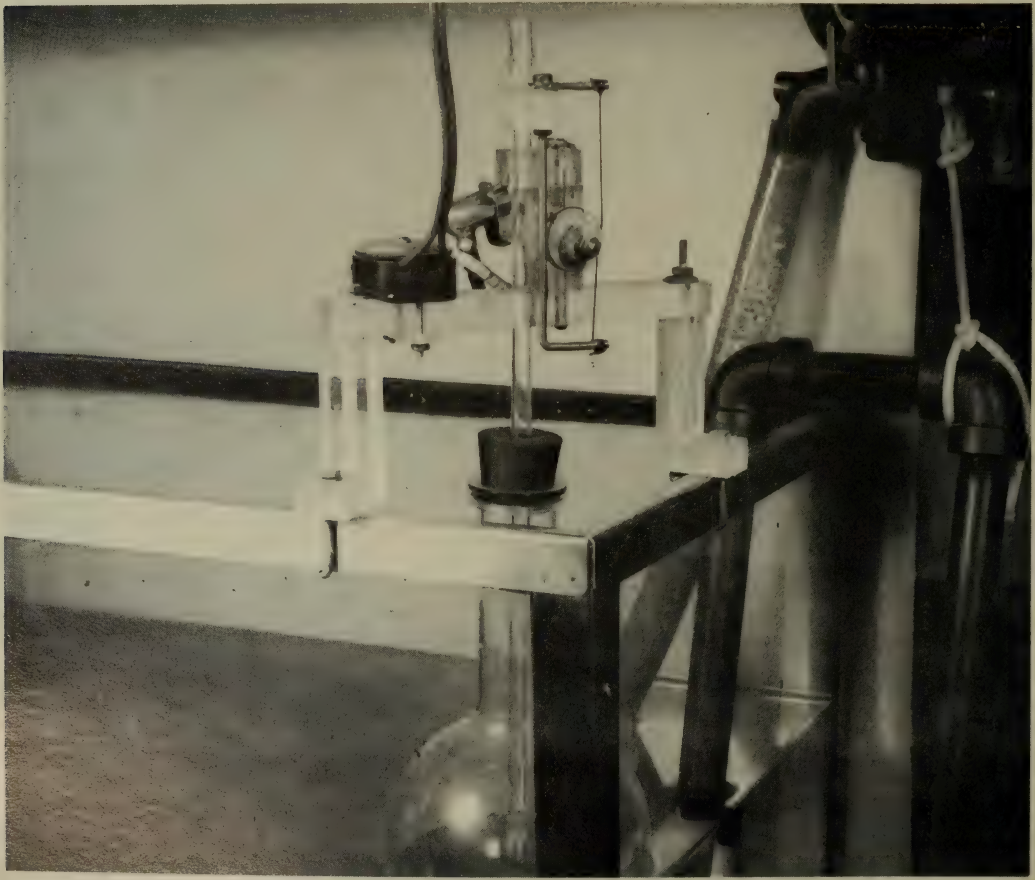

Figure 9.—Safety control for closed circulation. Its relation to the system is indicated in figure 8. At the right are the pump intake and excess return.

which operated in conjunction with the protective switch. This supply was administered by a float valve identical with that shown in figure 3. A very nice adjustment was found possible with these two float-actuated mechanisms, so that the dripping from the float-valve supply became directly proportional to the evaporation, without at any time tripping the protective cutoff float valve. On very humid days it could be seen that the number of drops per minute was notably less than on a clear dry day when evaporation was high. In the case of salt-water aquariums this device had an added important application which is discussed under the treatment of salt water.

Because of the nature of the controlling devices above described, it is necessary to observe certain details in starting the system. The levels of water in the lower series of aquariums will be different when the pump is not running than when it is in operation. This is mostly because the drain lines of the upper series of aquariums empty themselves into the lower aquariums when the pump is stopped. Therefore, the water is carried at a lower level in these aquariums so that there will be no overflowing when the circulation has been stopped. For this reason a switch should be shunted around the cutoff float to be used in starting the system before the operating level is reached. It will not suffice to wedge the float into a position where its switch will be closed, because its free action is necessary to establish its proper level of operation. After a dynamic equilibrium has been achieved by adjusting both the cutoff device and the density-control device, the shunt switch should be opened, after which the system should control itself. If it does not at first, very obvious adjustments of either or both will bring them into the proper relation.

The diagram of the closed marine circulating system shown in figure 8 employed seven aquariums for holding experimental fishes, only three of which are shown in the illustration. Three "reservoir" aquariums were used, of which only two are shown. These were standard commercial aquariums measuring 2 by 1 by 1 feet. The two smaller, used as filters, measured 10 by 8 by 6 inches. The pump was driven by a 1/10-horsepower motor and was rated at 10.8 gallons per minute at zero head. The pipe sizes are not indicated, as they would naturally vary with the needs of each system. In this one, the flow was slow but sufficient at about 3 gallons per hour through each of the seven top-row aquariums. At the right of figure 8 the supply pipe is extended upwards for some distance and with the upper end open. This permits building up whatever head of water is desired without subjecting the pipe to pressure greater than that produced by gravity.

Although the upper series of aquariums were intended for holding fishes and the lower series were regarded as treating reservoirs, the latter too may be, and have been, used to hold fishes, that is, all but the one from which the pump draws water, since the suction and turbulence here would be destructive to most small fishes. Aerating stones and a standard aquarium glass heater and thermostat comprised the rest of the water-treating equipment. The heater, which turned off when the water reached 74° F., was sufficient to keep the water throughout the system close to that temperature as it was only slightly higher than the normal room temperature. It was found that the aerating stones made it possible to permit the flow of water in the lower aquariums to run through submerged pipe outlets and thereby reduce the amount of splashing and consequent salt deposits. This was not found necessary in the upper series, for each supply pipe carried only one-seventh of the flow in the lower pipes.

MATERIALS

It is strongly recommended for all the purposes for which these devices were developed that only hard rubber or some biologically inert plastic be used. In fresh water, iron plumbing is adequate for many purposes, but for sea water no metals whatever should be used if any degree of satisfaction is to be obtained. Hard-rubber and acrylic-resin or vinyl chloride-acetate copolymer plastics have been used throughout for those parts which come in contact with the water, including the pumps. Also it is important to see that no brass or other such metals are used in positions over the aquariums in order to prevent possible corrosion falling into the water.

In all cases involving the use of pumps for aquarium purposes it is best to have a spare standby duplicate pump and motor unit as a precaution against the failure of either motor or pump. It is then possible to change such a unit in a few minutes, in the case of accident, with no serious interruption to the operation of the system. It is most convenient to use flexible connectors between the pump and the rigid plumbing leading to the aquariums. It is then necessary only to unfasten two screw clamps and insert the new unit in place. This type of arrangement is shown in figure 7.

An additional advantage of this kind of connection is that it dampens any vibrations from the pump or motor, which tend to travel throughout the system along rigid connectors. The pump in figure 7 was suspended by four light cords, a means which is also very effective in quieting such small machines.

TREATMENT OF WATER

The treatment of fresh water for aquarium purposes is too well known to warrant comment in present connections and is usually necessary only under special situations. The maintenance of sea water in a satisfactory condition is quite another matter. It is not the purpose here to discuss the theoretical aspects of the chemical and physical conditions of sea water. Such matters may be found extensively treated by Sverdrup, Johnson, and Fleming (1942) and Harvey (1955). The following is intended purely as a guide for the practical application of principles which have been found adequate to maintain a variety of marine fishes. Under this treatment regular reproductive behavior was quickly established in both *Histrio* and *Bathygobius*, which had been reared from juveniles. It also permitted a variety of volunteer algae and microorganisms to establish themselves. Incidentally these aquariums were kept under conditions of no daylight, the illumination being supplied by fluorescent tubes of the "warm white" type necessary for satisfactory plant growth. The periods of light and darkness were controlled by a time switch.

The equipment found necessary for the control of sea water consisted of a small hydrometer, a colorimetric pH device, and some simple titrating equipment.

The filters were provided with bone charcoal, and the bottoms of the aquariums and reservoirs were floored with so-called coral sand, and aerating stones were placed in various convenient places, but not in the aquariums containing fishes.

The specific gravity and pH were taken every day until the rate of change was established and from then on were taken at less frequent intervals. This rate of change will vary with the quantity of water, the bulk of the organisms contained, and the temperature of the water. At less frequent intervals titrations were made to determine the variously called excess base, titration alkalinity, or alkaline reserve. This method, which measures the bound CO_2, is not especially accurate but is sufficient for the present purposes. It consists in titrating a sample with N/100 hydrochloric acid to which brom-cresol purple has been added as an indicator. After the purple color has vanished, the sample is repeatedly boiled and further titrated until the purple color no longer reappears on heating. If the sample consists of 100 cc. to which five drops of indicator have been added, the final burette reading in cc. multiplied by 0.1 gives the bound CO_2 or bicarbonate in millimols/liter. This method is not to be generally recommended for accurate work but is sufficient as a comparative measure of how far and how fast the aging water is departing from its original value.

With this information, corrective measures may be taken. The specific gravity is nearly taken care of by automatic means involving the use of the float valve already discussed. Under normal operations distilled water is used to make up for the evaporation of sea water, which of course tends to increase its density thereby. This has been satisfactorily supplied from a 5-gallon carboy on a shelf higher than the float valve. The operation of the float valve holds the amount of water in the system at a constant volume, which means also that the dissolved salts will remain at a constant amount. If it is desired to increase the density of the water, instead of using distilled water as an additive, sea water may be used until the specific gravity has reached the desired level. If it is desirable to reduce the salinity, water may be withdrawn from the system while distilled water is used in the float-valve supply. This may be conveniently accomplished by means of a siphon with a small hose clamp so that the flow is restricted to a drip slow enough to permit the float valve to follow. Although distilled water was customarily used, in its absence tap water was used with no detectable effect on the fishes or the system.

If the pH falls to lower values it may mean that there is an increase in the amount of free CO_2 present. This could indicate too many organisms for the volume and temperature of the water or too much decomposition for the antacid components of the system to dispose of rapidly. The calcium carbonate in the sand should react with the acids formed, and unless there is overcrowding this type of decreasing alkalinity usually does not present a problem. If the placing of fresh activated bone charcoal in the filter results in an abrupt increase in the pH, it is almost certain that there is too much free CO_2 present. The use of charcoal renewed at short intervals will bring the CO_2 content down, but the charcoal rapidly becomes saturated and cannot be thought of as a regular part of the regulatory process. An increase in the number of aeration stones or amount of air they pass, while much slower in its effects, is a much more satisfactory way to insure against the accumulation of CO_2.

If, on a falling pH, none of the procedures above mentioned increase the pH significantly, the titration reading should be carefully checked and it too should show a decrease. This would indicate a lowering of the bound CO_2 which does not normally occur in an unoverloaded system in the presence of calcareous sand. If it does, however, more sand may be added, or sodium bicarbonate may be dissolved and administered with the distilled water through the float valve. Since the sand alone tends in a long-term sense to disproportionately increase the Ca in solution as compared with the Na, the occasional use of sodium bicarbonate, which tends to do the reverse, aids in keeping these two quantities in more nearly normal proportions. See Breder and Smith (1932).

The described procedures may seem to be somewhat complicated, but they are, in fact, not much more complex than those involved in maintaining a similar number of standing fresh-water aquariums. After the equipment is built and regulated, so that valves and controls are in balance, there is nothing to be done with them at any time, and in fact there should be no tampering with them at all. It is probably wise to post warnings to this effect. There is little aquarium cleaning to be done, as most of the accumulating detritus is automatically deposited in the filters. Aside from feeding the fishes and sometimes cleaning algae off the glass sides, the latter being controlled by adjusting the lighting arrangements, there are the following routine matters to be done. These will vary with each installation but may be approximated by the regimen under which the described installation of seven aquariums were controlled, as follows:

		Minutes
Read pH and specific gravity.	Twice a week	5
Titrate sample	Once a week or less	15
Fill distilled-water carboy.	Twice a week	5
Clean filters	Once in 2 weeks	15
Make the adjustments based on above information.	Once a month or longer	Various

The need for changing the pump and motor is such a rare occurrence as not to figure in the above schedule and should not take more than 5 minutes. Every attempt has been made to reduce the maintenance of the system to its minimum. It is not uncommon for the system to be left alone for as long as 3 days, as over a long weekend. The only thing to normally expect at the end of this period is some extra-hungry fishes. It should be borne in mind that the smaller the system—that is, its total amount of water—the more rapidly decomposition or contamination can spread through it. It is prudent not to reduce the size of the equipment more than necessary.

ADDENDUM

Since this article was first published the following additional data have been obtained.

Control of inflow

Since stopcocks with Teflon, or similar, plugs have become available, it is possible to simplify greatly the construction of small float valves. As these newer type stopcocks are usually provided with rather heavy glass tubes, no special support is needed, the glass tube being quite strong enough to support the float with the tube fastened rigidly only at the inlet side.

The only modification necessary is the removal of the usually colored handle in the Teflon plug. This is easily forced out of the hole in which it rests. It is replaced by a suitably sized rod, such as that shown in text figures 2 and 3. This rod carries the float assembly and connects it with the valve plug. As these valve plugs are held in place by a Teflon nut, there is no need for modification or construction of a holding frame such as that shown in text figure 2. Incidentally, these plugs are not liable to stick, even after several years of service.

Constant-level siphon

The constant-level siphon illustrated in text figures 1 and 3 can be made virtually foolproof if the open end of the glass tee, which faces upward, is extended by a piece of flexible tubing. This piece should be bent over so as to form an inverted **U**, with its open end facing down, into the aquarium. This is most easily accomplished by providing a third hole in the supporting crosspiece. The open end should be placed slightly above the level of the outflowing water in the horizontal arm of the tee.

A siphon so arranged will prevent overflowing of the aquarium even if the incoming water is increased over a considerable range. This is because, as the water rises, it occludes the open end of the downfacing tube. There is a slight negative pressure in this tube caused by the outflowing water in the constant-level part of the siphon. Thus, as the water rises in this accessory tube, the whole system becomes a simple siphon with two inlets, obliterating the constant-level feature. This increases the flow considerably, the amount being related to the length of drainpipe attached to the horizontal arm of the tee. The water level in the aquarium thus falls rapidly and the end of the inverted U tube is reexposed and the system regains its constant-level feature.

In practice, if the inflow of water to the aquarium is slightly more than the constant-level siphon will carry off, the water level in the aquarium will be at the mouth of the open tube. This tube will normally suck water from the surface and air, through a considerable range of inflow. Incidentally, this condition effectively removes any scum that may form on the surface.

The basis of this item was received from a correspondent shortly after the appearance of the 1957 issue of this paper. Unfortunately, the name of the individual concerned is no longer available, and it is therefore impossible to credit him properly here by name.

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MARINE AQUARIUM PROCEDURES AND TECHNIQUES

By Yves B. Plessis, *Assistant Director*

National Museum of Natural History, Paris, France

Abstract.—A closed-circuit system is used as the most dependable means of maintaining a balance of physical and chemical factors with a minimum of attention and maintenance. Several appliances as adjuncts to this system are discussed in detail, e.g., exhausting devices and capillary siphons which produce artificial tides. A special habitat for the breeding of intertidal animals in the laboratory, and a sand-bottom tank, designed from an esthetic point of view for public exhibition, are described.

Our present contribution to the aquarium sciences is concerned specifically with the field of experiment. The techniques that are already known, but which will be enhanced by details based on our experience, and the new, more important elements that make it possible to produce complex artificial environments will—we hope—be of service to the investigator as well as to those who maintain aquariums for exhibit purposes. The milieux that we have created for experimental uses have their place behind the scenes of a large public installation; they may also have their place amidst the tanks shown to visitors provided that they have been adapted and modified for that purpose.

DESCRIPTION

Being far away from the sea, we have been forced to use the same water over and over. It is not possible to obtain a biological equilibrium in an aquarium except by allowing the physicochemical factors as little variability as possible and, in any case, as is compatible with the survival of the population. The greater the volume of the water, the slower the changes occur and—in a general way—the easier it is to compensate for them. Moreover, a closed circuit with a large capacity is the most dependable means of keeping the desired balance.

There are several models of breeding installations that are adapted to the needs of marine biology. Most of them, including the one described here, have a reserve tank, a water circulation system, breeding tanks, and a filtering system.

RESERVE TANK

The reserve tank may consist of various materials but must have all of the following properties: Strength, water-tightness, and neutrality toward sea water.

A glass tank is relatively neutral but, unfortunately, is fragile. If it is large, stresses, changes of temperature, or vibrations may cause its destruction at any time.

If the tank is of concrete and well made, it has the advantage of being strong, but frequently it cannot be moved. Liquid that comes in contact with fresh concrete

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This paper was prepared in French; it was translated into English by a commercial translating service.

undergoes a very strong alkaline reaction which makes the use of concrete impossible unless it is first treated. Generally, prolonged washing with fresh water is not sufficient, and frequent rinsing with sea water is impossible when the distance from the sea is great. The best technique consists in covering the cement with a neutral product: special paint, paraffin, plastic substances, etc. In our installations, paraffin has given the best results; it was heated and spread with a brush and afterwards impregnated by the flame of a blowtorch.

The lining of cement with plates of glass is a very good method for large installations. Finally, the application of plastic materials practically solves the problem. At this time, there exist ready-made tanks of 100 to 500 liters, of plastic that is absolutely nonreactive to sea water. This technique is excellent.

WATER CIRCULATION SYSTEM

Once the tank has been installed, it is essential that the breeding tanks, which are generally located at a considerably higher level, be supplied. At the present time, there are rotary pumps made of plastic which are immersed in the water and operated by a shaft which plunges into the water and is driven by an electric motor. This device makes it possible to supply a reserve tank which, by gravity, supplies the breeding tanks. It is preferable to any other device when a number of persons use the installation. When the reserve tank is full, it is sufficient to open the desired faucet when water in one or the other tank is needed. There is one difficulty, however: the pump cannot operate continously. It is therefore necessary to provide an electric regulator, and that introduces a possible cause of failure.

Exhausting devices

The system we have used is much less cumbersome; it is based on water lifts which operate by means of air pressure, viz., exhausting devices. These are vertical pipes immersed into the water of the reserve tank; their upper ends emerge in the form of swannecks in the highest tank of the installation. Air supplied by a small electric pump is conveyed into the tube at a sufficiently low level within the reserve tank to move the water in the tube and make it ascend into the upper tank.

Our experience has led us to the formulation of certain estimates which save much guesswork. The interior diameter of the main body of the exhausting device must measure close to 6 mm. The pipe may be rigid or flexible; it may be of glass, of vinyl, or of polyethylene. It may be straight or bent, but must not have the properties of a siphon.

The air duct must open into the tube in which the water ascends, below the level of the water of the reserve tank, and it must be moved closer to that water level as the height of the exhausting device above the reserve tank is increased (contrary to what one might be tempted to do). As a matter of fact, the thrust of the ascending air pulls a mass of air above the level of the reserve tank, and that mass of air tends to become smaller as the air arrives at a higher level. It is necessary for the operation of the apparatus that the water mass above the level of the reserve tank exercise a vertical downward pressure from above, which must be less than the upward thrust that the height of the water in the reserve tank applies to the lower part of the pipe. In figure 1 we see the exhausting device, represented by the pipe AOB. AO is the upper part and OB is the lower part of the device; $O'O''$ is the water level in the reserve tank. It is quite evident that the influx of air at C will drive a water column CO in the direction of A. The farther C is from O, i.e., from the water surface, the larger will be the column CO. As soon as this water column

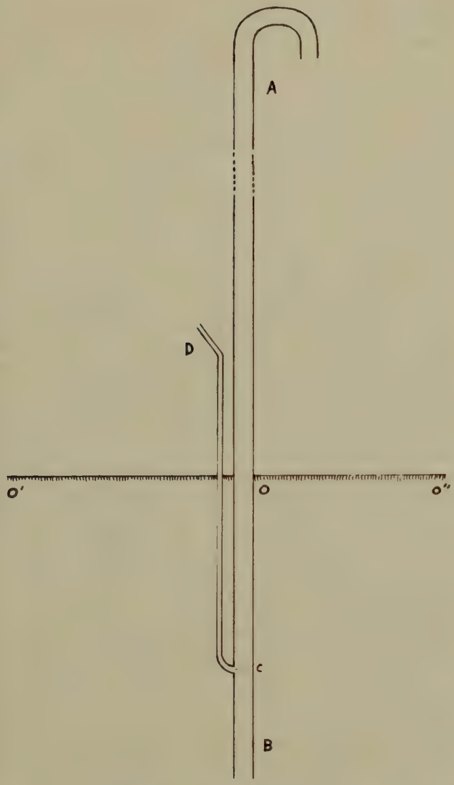

FIGURE 1.—General scheme of an exhausting device. Air arrives through tube *D* and ascends through tube *BA*; in doing so, it pulls water along with it.

starts moving, the water pulled along by the air bubbles joins it. The entire mass of water then exercises a downward pressure. The air which continues to reach *C* will be driven back toward *B*, and its pressure will increase until it reaches a maximum that is equal to the pressure exercised by a water column of the height *OB*. The device can function only when the pressure exercised in that way exceeds the thrust of the water mass contained in the part *CA*. In practice, an apparatus of this type is not used if it functions only with such intermissions as are due to the variations of the pressure at *C*. The height *OA* does not exceed two thirds of *OC*, and *CB* is very small.

It is advantageous to study the borderline cases; they have only a very weak output but are sometimes useful to know. A well-regulated apparatus, which works under the best conditions, has an output of more than 6 liters per hour. The necessary air pressure is a function of the height of the water *OB*. The pressure utilized under the most favorable conditions is theoretically slightly higher than the pressure of a water column of the height *OC*.

We have stressed the theoretical aspect of the problem of the exhausting device, since in practice the apparatus is susceptible of many variations in form, and since the conditions under which it is used and its perfection before use prevent a great many failures.

We have built most of our exhausting devices with glass tubing. A straight glass pipe 6 to 8 mm. in diameter ends at its lower part in a vinyl tube which is 10 to 15 cm. long and has an inside diameter large enough for the glass pipe to be inserted (fig. 2). Its bottom forms a strainer and is chamfered; it is perforated by small openings so as to prevent the deposition of sediments that may occur at any time. As near the glass pipe as possible, a hole has been made in the vinyl for the insertion of the end of the compressed-air pipe. This is a vinyl tube of small diameter. Plastic rings make it possible to keep it joined to the glass pipe for part of its length.

The upper end of the exhausting device is curved; it can be made in one piece extended and joined by a plastic ring.

BREEDING TANKS

In each one of our installations, the breeding tanks are made of molded glass. We have used several models the sizes of which correspond to the various populations.

Size of tank (length x width x height)	*Occupied by—*
15 x 10 x 15 cm	Scyphistomata, small groups of algae, Amphipoda, etc.
20 x 15 x 20 cm	Suitable for a very large number of animals of small size.
30 x 22 x 24 cm	Small Decapoda, mussels, Ophiuroidea, etc.
25 liters and above	Fishes, crabs, etc.

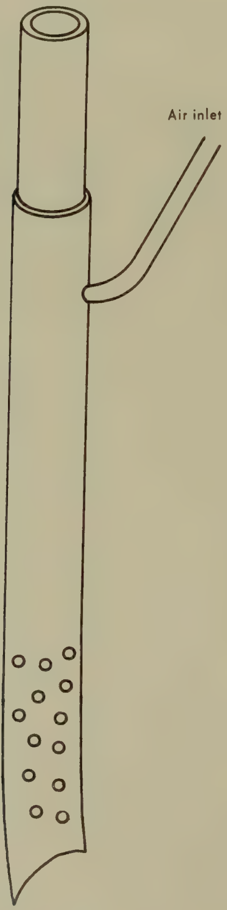

FIGURE 2.—Bottom of the exhausting device; it consists of a tube of plasticized vinyl. Its lower part is chamfered and perforated by little holes. *A*, air inlet.

Today's techniques make it possible to build tanks of transparent plastic materials. The main advantage is the possibility of having tanks built according to the model chosen, and of fixing water inlets and outlets without the need for any siphons. The fragility of these tanks is usually rather great, particularly in the large sizes. They must be well constructed so as to prevent the pressure of the water from tearing the seams of the walls apart.

Tanks with metal corners have the great advantage of being able to be built in large sizes. The metal must be particularly carefully insulated so as to prevent it from being attacked by the sea water. The plastic or bituminous linings that may be used are evidently unsuitable for physicochemical investigations of the water.

There are tanks of 100 to 200 liters' capacity, the frameworks of which are of fiber or cement covered with plastic. The only real inconvenience is the size of the framework.

In summary, in a particularly detailed study where the chemical elements have been specially studied, it is preferable to use only glass or plastic material.

Water supply to the breeding tanks is by means of a cascade arrangement. An upper tank receives the water from the reserve tank through an exhausting device or through a pump, and the excess flows into a tank located at a lower level, and so forth, down to the lowest one, which generally empties itself into the reserve tank by means of a filter.

Whenever the arrangement of a tank makes it possible, we have attempted to establish a cross circulation in such a way that the entire water mass is renewed. When there are only very small animals or plants, and when the current of the water is weak, the water may very well pass from the inlet to the outlet without renewing the contents of a tank. In that case, calm zones come into existence where the physicochemical conditions of the milieu may be very different from those prevalent within the current of the water. We may cause the water to arrive from the tank at an angle and at the bottom, while we cause it to leave at the opposite angle and at the top. If the current is sufficiently strong, and if the form of the tank is adequate, then a rapid test performed with a few milliliters of colored water—e.g., by the addition of a few drops of a neutral red or any other nontoxic coloring substance—will enable us to find some eddies, the localization of which can be most useful in a breeding tank.

Of the measures required to keep the water supplied with oxygen in the different tanks of a series, we have demonstrated a reduction of that oxygen which progresses from the exhausting device to the lowest tank. To remedy that shortage, we have arranged the tanks, not in a parallel series, but in the shape of a fan; this has enabled us to use progressively larger tanks. Accordingly, in the first tier each tank receives the water from one exhausting device. For example, four tanks receive each the water of one exhausting device. The second tier comprises only two tanks, of a larger size. Each tank receives the water of two higher tanks, that is, of two exhausting devices. The third tier comprises only one large tank which receives the water of the two intermediate tanks, that is, of four exhausting devices.

This distribution affords greater safety to the overall installation. As a matter of fact, if one exhausting device stops operating for one reason or another, only one single tank will not be fed any longer; the tank of the second tier that is branched upon it will still receive the water of the exhausting device of the neighboring tank while the tank of the third tier will receive the water of three exhausting devices. Finally, it is possible to double the number of exhausting devices and to feed them by means of two pumps both of which are branched upon all the tanks.

Connections between tanks—the siphons

When plastic tanks are used, it is very easy to make an overflow pipe by perforating the wall of the tank and attaching to it a plastic or glass tube. In the latter case, it is preferable to solder it. Any wood or rubber connections should be avoided. The former are easily gnawed by animals—sea urchins, gastropods, etc.—while the latter are frequently attacked by the sea water. It is easy to secure plugs of plasticized polyvinyl chloride; they remain flexible and are not attacked by sea water. (Note well: some polyvinyl products are not neutral in sea water.)

When the breeding tanks are made of glass (our installation is of this type), the perforating of the walls is always a delicate operation. The use of siphons makes this operation unnecessary. Over a period of several years, we have used two types of siphons which have been entirely satisfactory:

1. *Permanent siphons.* — Generally speaking, the siphons will not run dry as long as the inlets and outlets remain in the water. In the siphon shown in figure 3, the inlet A is maintained constantly within the water by means of the mounted swanneck C which connects directly with the outlet of the siphon. An inlet for air has been created at E to simplify priming the siphon between B and D. On the other hand, that air inlet makes it possible to prime the first siphon by aspiration through that opening with the aid of a flexible tube. The water arrives through A and F, filling the tube. When the flexible tube is withdrawn, the part CD will dry up by itself, and the apparatus will be in operating condition. When the end F does not reach into the water of the lower tank, it is necessary to plug it during the priming operation.

We have equipped the inlet of certain siphons with a sort of funnel, the opening of which is covered by a nylon screen that

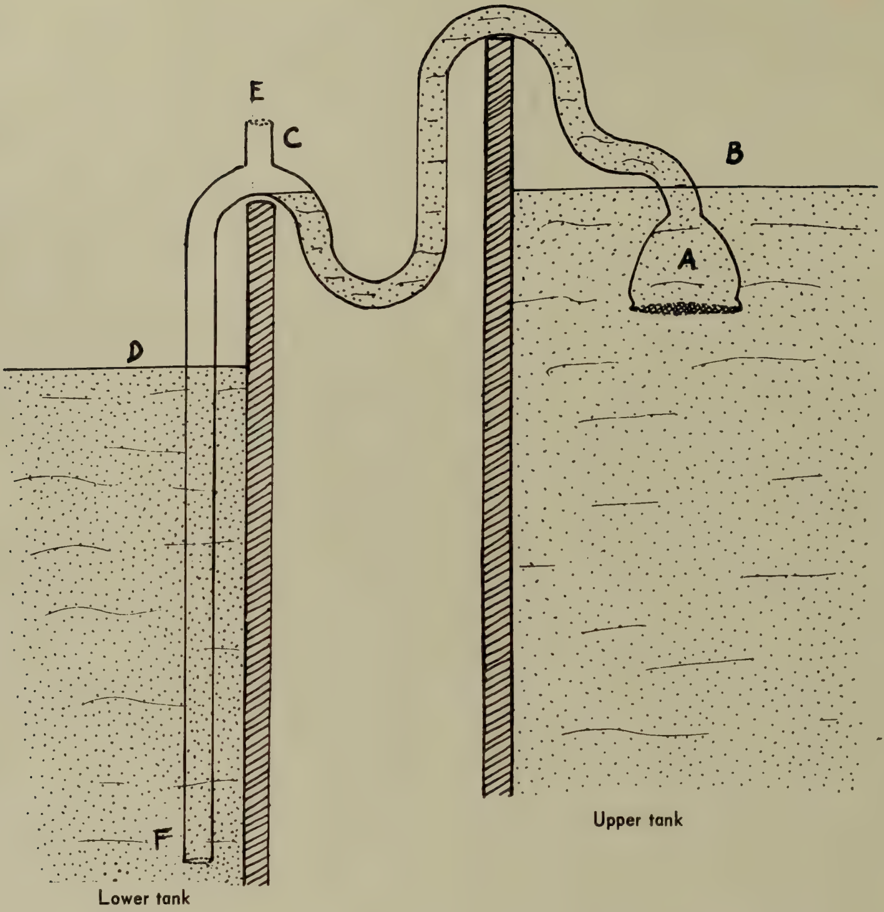

FIGURE 3.—Type of permanent siphon; *A*, flared opening of the siphon, capable of accommodating a screen; *1*, upper tank; *2*, lower tank; *E*, air opening.

is fixed there by means of a vinyl ring. This has enabled us to separate, from one tank to another, very small animals which, without that precaution, would have passed through the siphons. These funnels are preferably positioned in such a way that their opening is at the bottom end and as far away as possible from the walls of the tank so as to reduce the risk of the deposition of sediments.

The level of the upper tank is regulated by the height of the swanneck *C*; the level of the lower tank is determined by its outlet siphon which does not appear in the drawing.

It is helpful to have a supply of siphons of this type. In order to clean them, it is sufficient to place them, from time to time, in a hypochlorite solution to soak, and to rinse them well before use. When the siphons do not have screens, a sulphochromic mixture is preferable.

2. *Capillary-priming siphons.*—We have perfected a siphon the curved part of which is so narrow that capillarity may be able to prime the system even before the water reaches the point of overflowing (figs. 4, 5, 6, and 7). By duplicating our piping system of permanent siphons with

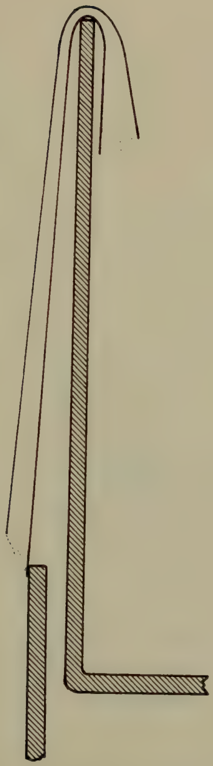

FIGURE 4.—Theoretical section of a capillary priming siphon flaring at both ends.

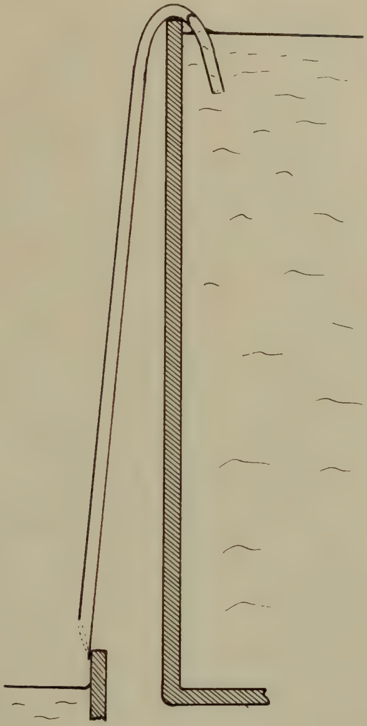

FIGURE 5.—Simple siphon, poorly made (lower part is too big). It is essential that the lower part of the siphon is not immersed into the water.

these capillary siphons, we have put into effect a very simple and highly efficient safety device. In order for such a siphon to be able to begin its priming action, it is essential that its lower end does not reach into the water, so that the water of the upper tank may ascend freely in the tube, and that—aided by capillarity—the water may reach the highest point of the curvature before the water level in the upper tank goes past the edges. When a system of this type operates within a tank, it introduces continuous variations of the water level into it, provided, however, that the supply to the tank is much smaller than the output of the siphon, and that the inlet of the siphon has a much larger diameter than its curvature.

We have also used a siphon the upper part of which is capillary; in that case, the siphon no longer exercises practically its priming action, and the water level in the upper tank remains constant. Capillarity keeps a small quantity of water in the tube of the siphon; when the supply to the tank becomes weaker than the output of the tube, this water breaks up into short water columns whose distance one from another becomes greater as the water supply in the siphon becomes weaker. This siphon practically never runs dry.

In these siphons, the current of the water is strong, and the organisms, particularly the Diatoma, attach themselves less rapidly there than on the permanent siphons where the current is weak. But inasmuch as they serve as a subsidiary canalization, the pipes are normally not operating, so their maintenance is limited to cleaning them after an occasional oper-

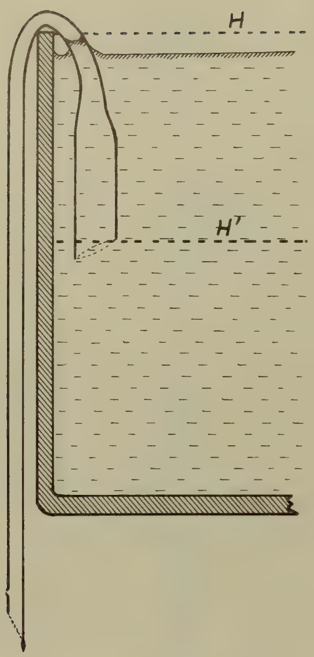

Figure 6.—Intermittent siphon with capillary priming, well made: *H* and *H'* represent the range of the "tides."

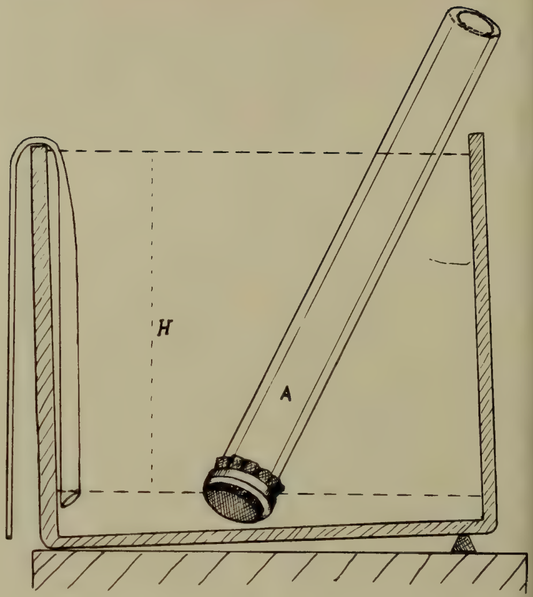

Figure 7.—Scheme of a breeding tank for very small animals: *H*, range of "tides"; *A*, tube into which the little animals are placed.

ation. Consequently, they are always ready for use.

On the other hand, the establishment of a siphon for capillary priming is not very easy, and the satisfactory operation of the system is dependent on a certain number of favorable elements:

1. The curvature of the siphon must be smaller in the downward direction than in the upward direction. The diameter of the tube must be slightly smaller at the highest point so as to facilitate the priming. Any other arrangement will adversely affect the satisfactory operation of the system.

2. The bottom of the siphon must be made in such a way that there will be no hanging drops. A hanging drop would act as a plug and prevent the water from ascending, by capillary action, into the upper part of the system. We have therefore attached, at the outlet of the siphon, either a flare designed to prevent the hanging drop, or a chamfered "glass drop" at the end of the tube, which serves the same purpose.

3. The tube must be made of glass that can be wetted, and not of plastic. Sometimes it happens that after a prolonged use the tube remains dry for some time. In that case, it is almost always necessary to "wet" it in order to put it back into operation. A washing with a sulphochromic mixture is then required to make it as good as new.

The diameter of the tube, at its narrowest part, may vary on a wide scale, but it must be sufficiently large to enable a water current to drive the air out of the lower part of the siphon when the siphon is formed by a tube which has an interior diameter of 6 mm. and is tapered in the curvature. When the siphon is formed by a small tube having an interior diameter of 3 mm., the curvature need not be tapered, but in this case the lower part of the siphon must be elaborated most carefully so as to avoid the formation of hanging drops, since this last-mentioned system, while it rarely dries up, also cannot be put back into priming action as readily as the preceding system.

We have come to the point where we prefer to use the first type of capillary system, which dries up frequently and can be put back into priming action easily, in order to create "tides" in small glass tanks. This arrangement has enabled us to succeed with breedings which would have been difficult to achieve under other conditions. When the feeding of the tank "in the tidal way" in this manner is of too large a volume to have the tank emptied by a capillary siphon, we have been using a complex system in which the capillary siphon primes one or more simple siphons. This type of apparatus is rather difficult to build—if not to conceive—but once it has been completed, it is able to operate for a very long time without any surveillance. The simple capillary siphon, for tides, must have a very large inlet so as to dry up abruptly at the end of the operation, when the air is swallowed up into the tube, owing to the lowering of the water level in the upper tank.

Utilization of the "tides"

It must be mentioned at the outset that these artificial "tides" are only very distant relatives of the true tides which, from the biological point of view, are rather complex phenomena (currents and inversion of currents, change from an agitated to a calm phase, variations of the turbidity of the water, of its temperature, salt contents, luminosity, etc.).

The use of capillary siphons to create "tides" is not limited to the breeding of animals which one wants to flush out periodically. We shall now briefly review the uses to which we have put them, but it is possible to think of many other applications:

1. It is not always easy to breed small animals when it is desired to confine them within a restricted area for "boarders" (temporary occupants) which, at the same time, require very frequent changes of water. Into a "tidal" tank, tubes that are open at both ends may be placed vertically. As the water level is variable—from the bottom of the tube to its upper end—the volume of the water that passes into that tube during any given period is proportionate to the number of tides that have taken place during the same period. If it is desired to breed animals in these tubes, it is sufficient to block the ends by a nylon screen. In that way, the animals can be isolated easily, they are able to have enough water, and the breeding is limited to a very small area. In practice, the breeding tubes are assembled into bundles, and only their lower end is immersed into the "tidal" tank (fig. 7).

2. For breeding of animals within the upper limit of the tides (fig. 8), a tank (*A*) receives water from the exhausting device through a large pipe which descends to the bottom of the tank. A bed of gravel has been placed on that bottom; it is covered with a screen and with sand—an arrangement that is similar to the sand tank which we shall discuss later. The breeding tank proper (*B*) has been placed on the bottom of the sand layer; this tank consists of a glass cylinder which is closed, at its upper end, by a removable sheet of glass (*F*). Pebbles collected on the seashore have been placed inside the cylinder on a layer of sand the thickness of which varies but must be such that its level is very considerably lower than level *1*.

The level of the water in tank *A* varies between *1* and *2*, because of the siphon *C*. The water ascends and descends in cylinder *B;* it wets part of the pebbles which, therefore, retain a high humidity. The water in cylinder *B* acts amidst the pebbles like a piston, in such a manner that

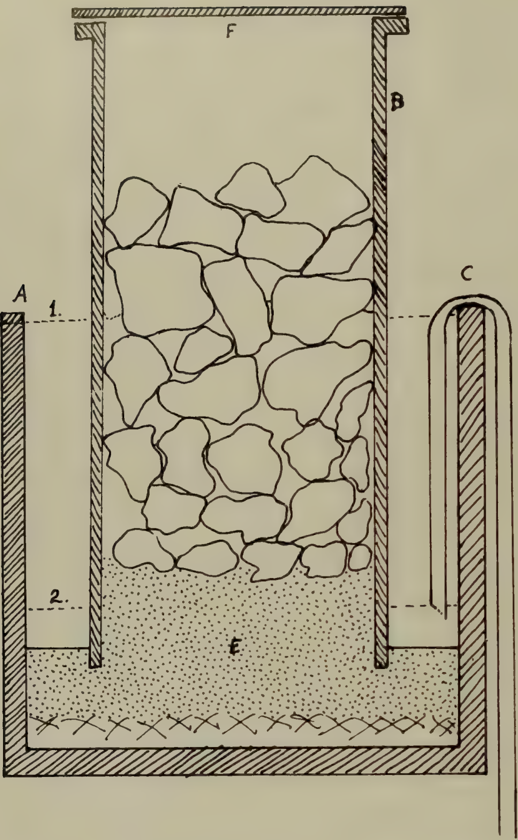

Figure 8.—The "tidal" tank (*A*) into which is immersed the bottomless tank (*B*) which rests on the sand (*E*). A plate of glass (*F*) covers the upper part. The air enters and leaves at the cover (*F*) without having to pass the small breeding animals. Since the saturated atmosphere is not suitable for some animals, it is sometimes necessary to replace the cover (*F*) with a fine screen, but in this case the pebbles of the upper zone are quickly covered with salt precipitations. *C* is an intermittent capillary siphon.

the air passes, without stopping, the edges of the cover *F* which has been simply laid upon the cylinder. In that way, the air in the breeding area is constantly renewed. It is advisable to provide a sufficient height so that the upper level of the pebbles will be relatively dry. It is also possible to replace the glass plate *F* by a fine screen.

3. We have also made use of the "tidal" tank whenever a breeding situation required good irrigation while the topography of the breeding tank did not afford it. Thus, when the accumulation of substances of all kinds in a tank is such that the circulation is poor, it is very useful to have recourse to this technique which makes it possible to renew the water, in its totality and everywhere, with every tide.

The substratum

Generally speaking, the breeding tank does not require any special substratum, and the glass or plastic wall of the tank itself constitutes the breeding ground.

If it is desired to provide rock animals with a suitable substratum, an adequate piece of rock is placed in the tank; generally, this presents no difficulties. Such, however, is not always the case when it is desired to introduce a sandy or muddy bottom.

An anaerobic milieu develops very rapidly under the first millimeters of the ground; it immediately begins to discharge hydrogen sulphide which may cause the death of the animals and plants to be bred. We have introduced a very simple solution to this problem.

Quantities of oxygen that were dissolved in the interstitial water of the coastal sand have shown us repeatedly that the partial oxygen pressure is weak: approximately 30 to 40 percent of saturation. In certain cases, the oxygen pressure is practically nil. These facts can be explained easily: the oxygen contained in the interstitial water of the sand has its origin in the irrigation of that sediment. When the sand is subject to the swing of the tides, its level also is subject to variations that bring about a circulation of the water. It is a matter of course that this level, for a certain amplitude of the tides, is subject to variations which increase as the elements of that amplitude become rougher. Another intervening factor is the action of the fauna which modifies the permeability of the ground by burrowing corridors. The fauna which consumes oxygen favors the circulation of water in the submerged sand. These considerations have permitted us to use substrata of sand of considerable thickness, capable of accommodating a rich fauna. We have built this tank with the thought in mind that an adaptation period is required during which the fauna and the flora become settled and establish a certain equilibrium; thereafter, the artificial balancing mechanism (water current, etc.) can be reduced considerably, without any important change of the biotope.

The scheme of an experimental sand tank is shown in figure 9. The water comes in at *1*, through the large vertical pipe which is open at *2* and *3*. The water reaches the bottom of the tank directly, flows freely between the pebbles and the various shells, passes through a nylon or fiberglass screen and filters through the sand layer *6*. The sheet of water *7* flows off through the siphon *8*.

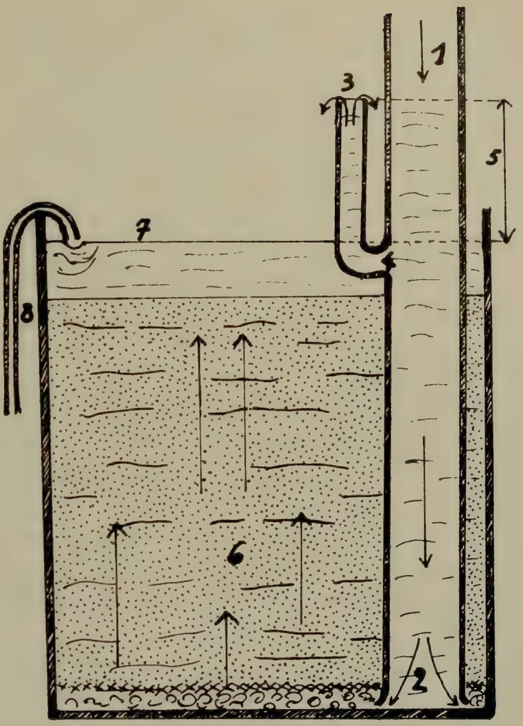

Figure 9.—Scheme of a sand tank: *5*, height of the water determining the pressure required for irrigation of the sand.

The volume of water which arrives at *1* does not flow off easily through the sand layer. When the water level in the large pipe rises the pressure in that pipe increases. That pressure must not be too high because the water would immediately pour forth violently from the sand, in the form of a "spring." It is easy to determine the optimum pressure. In practice, the height of the water column above the level of the tank (*5*) must amount to approximately one-fourth the depth of the sand. It is possible to make an opening of any size in the large pipe, preferably at that level. This is what we have done in the case of the usual breeding tanks. We have now begun, however, to make experimental tanks that have an opening *4* at a lower level than that of the water in the aquarium. To that opening, we have attached a bent tube which is open at *3*. By making that tube rotate around *4*, the opening *3* may now be brought to the level desired. By means of devices of this type we obtained our first data concerning the equilibrium of aquariums with sand bottoms. When the tank has operated in that way for a certain time, the sediment settles somewhat, the bottom becomes clogged, and the amount of water that will effectively pass through the sand on its way from the large pipe is reduced gradually. One may then remove the bent tube and, thereby, suppress almost completely the irrigation coming from the large pipe. The irrigation of the sand will then continue to be taken care of almost exclusively by the fauna that has been installed in the sandy substratum, and no symptoms of anaerobiosis will appear.

During the numerous tests which we made we had never made a lateral opening in the large pipe. We noticed, then, an inconvenience of rather secondary importance when this mode of procedure is adopted: the inflow of the water which most frequently is handled by means of an exhausting device causes precipitations of water that we later have tried to prevent. Nevertheless, one of our installations has made use of that very phenomenon to create a "spindrift zone."

The tanks with mud bottoms are conceived along the same lines as those having sand bottoms. It must be noted, however, that the irrigation of the mud by means of the water column of the large pipe is extremely weak. Almost all of the water passes through the overflow pipe of the large tube. Too strong a pressure on the mud must be avoided because the mud is much less resistant to the "spring" phenomenon than the sand. As to the surface, it is particularly important to study the water current so as to prevent its scooping out the mud. Finally, when building a tank of this type, it is advisable to provide, between the screen at the bottom of the tank and the mud, a layer of a few centimeters of sand.

FILTERING SYSTEM

The tanks with sand bottoms may be located at any point within the closed circuit of an aquarium. They may be mounted in series or parallel. It may be well to note that they act as complete or partial filters, and for that reason they should be intercalated at suitable points in the circuit. We have used these tanks as filters in several different circuits. They do not need any frequent maintenance operations and are able to go on for years. Generally, they act as partial filters, and the problem of complete filtration will have to be solved many times without them. We have not used any permanent filters but have been content with sand-bottom tanks acting as partial filters since—in a complex breeding installation where we have been more interested in bringing about a biological equilibrium than in the survival of a certain species—such a filter traps the plankton and we generally were eager to avoid such a trap. But in a breeding installation where microfauna and microflora are suspended in the water and constitute a true plankton, it is sometimes necessary to introduce a complete filter into the circuit in order to restore an equilibrium that has been upset by an intervention of an experimental nature. For that purpose, we have used a large-dimension Pyrex glass tube, having a diameter of 110 to 120 mm., and a length of 80 cm. It was placed vertically into the reserve tank where it exceeded the water level by 30 to 40 cm. Its lower part is closed by a plug of polyvinyl chloride which is perforated and is kept in position by plastic tubes that are reinforced internally by metal rods. These reinforced tubes are held at the upper part of the Pyrex tube by their ends which have the form of hooks. We introduced empty shells into the filter, at its bottom, in a layer having a thickness of 5 cm., followed by a nylon screen which holds a 20-cm. column of charcoal; this is followed by a thin layer of glass wool covered by small pebbles in a thickness of 15 to 20 cm. This filter is kept in place during the time required and is maintained at a suitable level.

MODIFICATION OF THE SAND MILIEU FOR A PUBLIC TANK

The installation with a sand bottom that has been described may be of great interest in a public aquarium for the presentation of psammophile anemones, pleuronectids, sand crabs, etc. In large sizes, this apparatus can be installed with two lateral edges that recede according to Garnaud's principle of the tank with nonparallel sides, and with plate glass inclined at about 20° from the vertical so as to show the sand bottom at a convenient angle. The back of the tank (the part opposite the glass) will be strongly concave and very much bell-mouthed. In this section, the wall will be inclined at about 45°. Under these conditions, an observer looking through the glass will not see the lateral edges; he will practically not see the water surface, and he will easily confuse the back and the floor.

The principle of the aquarium with nonparallel sides according to Garnaud has one fault: it presents to the public an exhibit area that is relatively small as compared with the size of the tank, and if the exhibit is particularly attractive the visitors will crowd each other in front of the aquarium. Consequently, we suggest an entirely new form in which two sides are slanted parallelepipeds that enable the visitors to see the exhibit clearly from two angles. The advantage in servicing the aquarium is certain: despite the large size of the tanks, they have very little depth (fig. 10, 11, and 12).

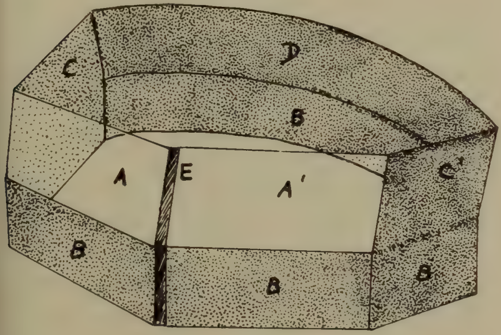

Figure 10.—Sand tank for public exhibit. The heavily stippled areas represent the blind sides of the tank. Sand fills the lower section (*B*) of the entire tank, up to the bottom edge of the plate glass (*A* and *A'*). The back of the tank (*D*) slopes backward in order to blend visually into the sandy soil bottom. *E* is the channeled corner holding the plate glass.

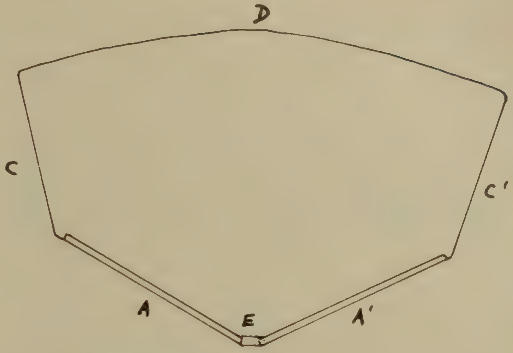

Figure 11.—Bird's-eye view of sand tank for public exhibit, showing 5-sided shape of tank.

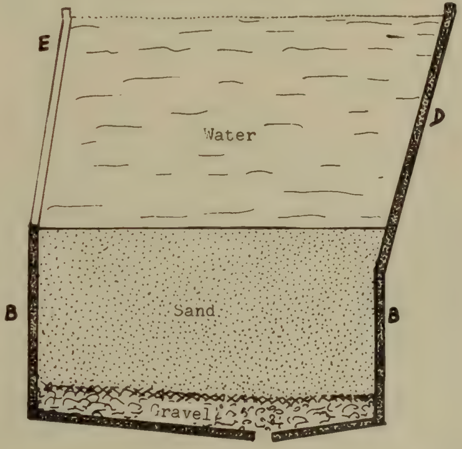

Figure 12.—Cross section through the middle of the sand tank, from *E* at center front to *D* at back.

An aquarium of this type, in which the length of the two panes of glass reaches 2 m. while its total length amounts to 2.50 m. and its width is 1.60 m., affords a field of view that appears to be unlimited. In this example, the height of the free water would certainly not exceed 1 m.

Cultivation of algae

This arrangement is very efficient for cultivating marine algae, either for particular studies or for maintaining an isolated marine environment in perfect equilibrium (figs. 13 and 14).

We have developed a technique of culture upon a film of water. Under these conditions the gaseous exchanges between the air and water attain their maximum activity. Most of the algae spread out and assume strange forms; *Codium tomentosum*, for example, develops a thick knobby cover. This technique for cultivation of algae raised on glass, commercially known in France by the name of "lessive," permits the complete isolation of volumes of air which are important for raising aerobic marine invertebrates separately upon a blanket of *Codium* sp.

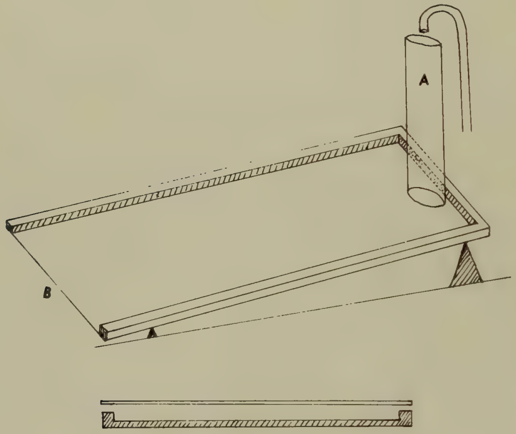

FIGURE 13.—General plan of equipment for cultivation of algae: *A*, inlet of water; *B*, outlet of water. The general plan without the top plate is shown at top; a cross section showing the top plate is at the bottom.

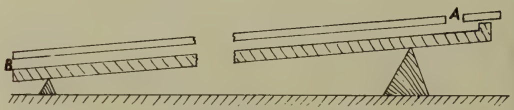

FIGURE 14.—Cut-away section of algae-cultivation equipment: *A*, inlet of water; *B*, outlet of water.

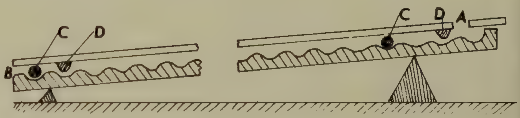

FIGURE 15.—Cut-away section of a plate of the "lessive" type: *A*, inlet of water; *B*, outlet of water; *C*, glass rod for breaking up the flow of water; *D*, bar of glass fastened to the top plate for breaking up the flow of water.

CONCLUSION

We have acquired our experience in "aquariology" by looking for special solutions to problems of experimental ecology. The rules that we have worked out gradually will perhaps enable others to save a good deal of guesswork. The actual establishment of special habitats is even now most helpful in the field of experimental investigations.

The needs of the public aquarium are different from those of a research aquarium where the care for the living conditions of the occupants is not combined with esthetic preoccupations. Many of the techniques described here have been conceived for the purposes of experimental ecology and cannot be transposed directly; on the other hand, the aquarium with a sand bottom—presented in a tank with nonparallel sides and with inclined glass—is certainly a fortunate transposition from the experimental scale to the large-scale public presentation.

A FAST-FLOW CLOSED-CIRCUIT MARINE AQUARIUM

By L. J. Hale

The Ashworth Laboratory, University of Edinburgh, Edinburgh, Scotland

Abstract.—A relatively fast flow of sea water results in a much greater success in keping marine organisms in aquariums, and a closed-circuit system will remain biologically balanced without changing or filtering the sea water and without temperature control. The water is actively circulated by a special pump which is an elaboration of the bubbling tube. This pump has advantages over conventional pumps in the absence of corrosion and contamination and of damage to organisms passing through it; it requires little maintenance.

In earlier papers (Hale, 1957, 1960a) I briefly described closed-circuit experimental aquariums which probably owed their success primarily to the relatively fast circulation of the sea-water. There were neither filters nor reserve tanks in the system; the water was never changed nor its temperature controlled. From the experience so gained a permanent apparatus was built and has been operated successfully for over 4 years. The details of this aquarium are described in this paper.

ECOLOGICAL CONSIDERATIONS

The essential features of this aquarium are based on possible changes in the aquarium water, and how these changes might be counteracted by providing an adequate flow of water. These considerations will be discussed briefly.

Inorganic ions

The greatest problems encountered with sea-water aquariums arise from changes in the concentration of ions which occur in the artificial environment. These changes are caused by the contained organisms, and any radical departure from normal is likely to be detrimental. Important among these ions is calcium. The concentration of this ion is likely to be rapidly reduced by the many organisms processing calcareous skeletons; they actively remove calcium from the sea water (Bevelander, 1952; Robertson, 1941; Wilbur and Jodrey, 1952). A decrease in the calcium ion concentration might also take place if the carbon dioxide tension were reduced by too much photosynthesis (Harvey, 1957), and also if the sulphate ion concentration rose as a result of aerobic putrefaction of excessive protein sulphur from dead organisms. Replacement of calcium is likely to take place if an excess of calcium carbonate is kept in the water, such as mollusk shells or limestone chips. It is also clearly an advantage for the pH of the sea water to be kept normal (8.1–8.2) by providing light for an adequate degree of photosynthesis. Photosynthetic plants are also desirable to control inorganic nitrogen.

The concentration of ions containing nitrogen is likely to increase considerably over the minute amounts contained in the sea (Oliver, 1957). Ammonia is excreted by many marine animals; the excretory urea of some will be rapidly hydrolyzed to ammonia. Ammonia is oxidized by nitrifying bacteria to nitrite and then to nitrate. It will accumulate as nitrate unless removed by photosynthetic plants.

Other major constituents of sea water are unlikely to alter significantly, but the concentration of some minor constituents may do so. For example, phosphate is removed by plants and some animals, and excreted by animals but information on this ion is too meager to make a useful assessment of the situation in an aquarium. Other ions, such as iodide, silicon, and copper, may become depleted when particular organisms requiring them are kept in the tanks.

Suspended particles

Marine bacteria are largely sedentary (ZoBell and Anderson, 1936); nitrifying bacteria are found associated with suspended particles. These particles may adsorb ammonia (Cooper, 1948a), thereby encouraging bacterial oxidation.

Most of the iron in the sea occurs as colloidal particles (Cooper, 1948b; Cooper, 1948c; Harvey, 1937b). Nitrifying bacteria require iron (Spencer, 1956), as also do diatoms (Goldberg, 1952; Harvey, 1937a) which are useful photosynthetic organisms in an aquarium. Suspended particles are essential as food for filter-feeding organisms. Thus suspended particles are an important feature of the marine environment, and the common practice of filtering aquarium water would appear to be harmful.

Water movement

The necessity for an active movement of water in an aquarium is indicated by the fact that the majority of organisms kept therein are relatively sluggish or sedentary. The materials they require for their existence which are contained in the water, and the desirability of flushing away excretory products, must therefore be mediated by the water. Marine organisms fall into three main groups as regards these materials. Firstly, there are the animals and the aerobic putrefying bacteria; they require oxygen, and their waste products include carbon dioxide, ammonia, and some inorganic phosphate and sulphate. Secondly, there are the nitrifying bacteria requiring oxygen, ammonia, and phosphate, and producing carbon dioxide and, ultimately, nitrate. Thirdly, there are the photosynthetic plants which, in the presence of light, more importantly require inorganic nitrogen, phosphorus, sulphur, and carbon dioxide; they give off oxygen. Thus the requirements of one group are the waste products of another, and a stream of water is the obvious means of transportation of these materials.

To this must be added the function of a moving stream of water in supporting suspended particles.

There may be other beneficial properties of an active water movement. For example, marine hydroids become moribund and die in 1 or 2 days in stagnant water, but they will live indefinitely in a moving stream of sea water (Hale, 1960a, 1960b). The reasons for this remain obscure.

In the aquarium here a rate of flow of water of 10 feet per minute is maintained and is generally satisfactory. Faster and slower rates are found in different parts of a tank. The flow may be increased considerably by the use of baffles.

Temperature

Consideration of the literature on the temperature tolerance of marine organisms indicates that a temperature up to at least 20°C. is not harmful to organisms from temperate seas. The water temperature in the aquarium here has risen to 21° C. without noticeable effect on the contained organisms.

DESIGN OF AQUARIUM

This brief survey of aquarium conditions points to a simplicity in the design of aquariums in that filter beds, temperature control, and large stocks of sea water (an important consideration to laboratories remote from the sea) are unnecessary. The degree of success of the aquarium here supports this conclusion. The tanks in this aquarium are arranged in series with a specially designed water-circulating pump placed between two tanks in the circuit. Thus the sea water passes from the pump through one tank after another and back to the pump again as a continuous circulation of the same water. The pump and tanks are designed so as to maintain an adequate flow of water; all parts of the apparatus in contact with the sea water are of glass or plastic. There is a device for producing tides, technically easy in a closed system, and lighting is provided for some tanks.

The pump

The desirable features of a sea-water pump are that it should not corrode in sea water nor should it have moving parts in the water. Corrosion shortens the life and reduces the efficiency of the pump. Metals (except perhaps stainless steel) may corrode and liberate ions toxic to marine organisms. Moving parts are exposed to abrasion by sand grains and may damage organisms passing through them. To overcome these difficulties the pump I have used is an elaboration of the well-known bubbling tube, and is fashioned in plastics. A bubbling tube consists of a vertical tube into the bottom of which air is pumped; the bubbles rise to the top carrying water with them. The efficiency of such a tube depends on its length and bore, the rate of air injection, and the head of water against which it pumps. Figure 1 shows that a tube of about ½-inch bore with a low rate of air injection is the most efficient. More water may be pumped by increasing the rate of air injection, but the efficiency of the tube decreases. The efficiency of a bubbling tube also increases if its length is increased. It decreases in efficiency as the head of water against which it pumps increases; this is not a serious objection for closed-circuit aquariums, as the head of water is never more than a few inches.

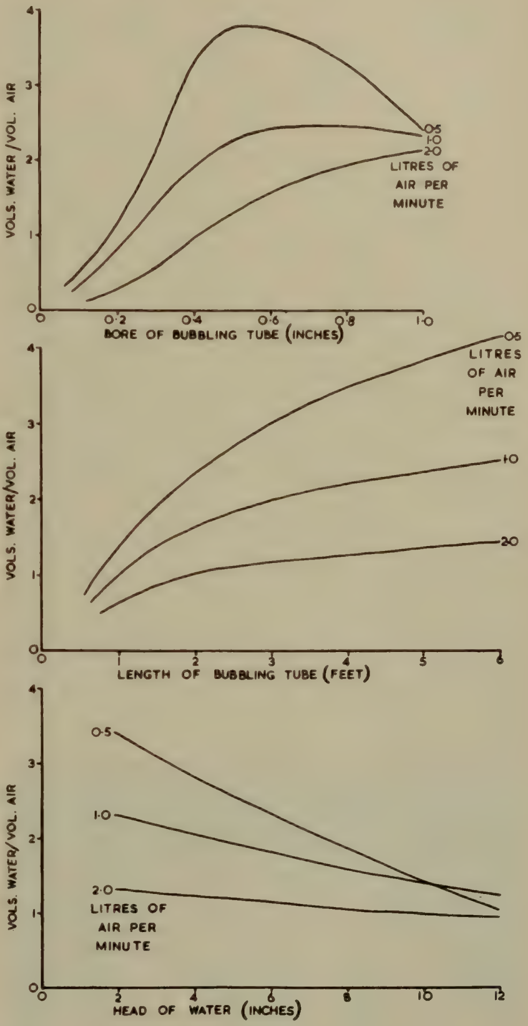

FIGURE 1.—Efficiency of bubbling tubes (volume of water per volume of air) at stated rates of air injection. At top, effect of the bore of a tube (5-foot length, no head of water); in the middle, effect of the length of a tube (½-inch bore, no head of water); at bottom, effect of the head of water (5-foot length, ½-inch bore).

The pump I use here is provided with 12 bubbling tubes of ½-inch bore. The base of the pump (figs. 2 and 3) is fashioned of Perspex. Water from the 'last' tank is led to the central 2-inch-diameter water inlet by a polythene tube from a similar adapter attached to this tank (fig. 4). The water then passes out into the ring of bubbling tubes. These are of PVC (polyvinyl chloride) and carry the water, plus air bubbles, through an adapter (fig. 4) to the 'first' tank. Air is injected into the bases of the bubbling tubes through the outer ring of nozzles; the size of the air orifice has no effect on the efficiency of the bubbling tube.

Each air inlet must have its own control valve. In the present equipment, air from a compressor passes into a Perspex chamber provided with 12 air outlets, each having a simple Perspex valve. It is possible that a simpler design would be to fit these valves to the basal part of the pump. The required pressure of air is that which is sufficient to overcome the pressure of the column of water in a bubbling tube (for example, ⅕ atmosphere for a 6-foot tube).

When working at an economical rate this pump moves about 250 gallons of water per hour (300 U.S. gallons) for the expenditure of about 12½ cubic feet of air. Increasing the rate of air injection increases the rate of pumping water but is less efficient; 25 and 50 cubic feet of air will move respectively about 310 and 375

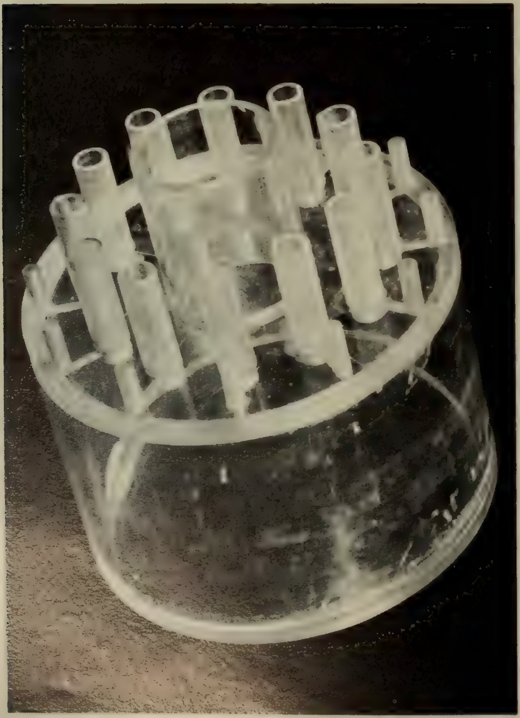

Figure 2.—Pump base, made of Perspex, with 12 PVC bubbling tubes of ½-inch bore.

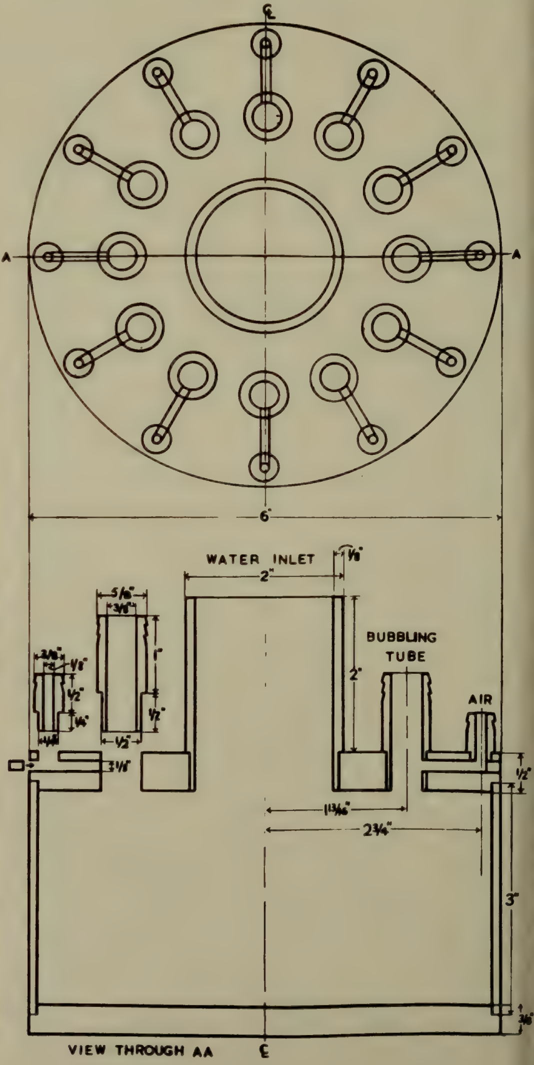

Figure 3.—Plan and section of pump base.

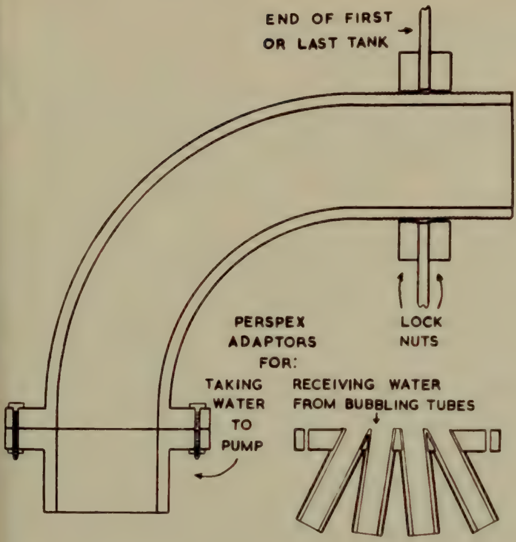

FIGURE 4.—Units for taking water to and from the pump. They are of black Alkathene except for the adapters which are Perspex.

gallons of water per hour (370 and 450 U.S. gallons).

This pump has run without fault for over 4 years; its only maintenance is an occasional cleaning (about twice a year).

It should be noted that the pump also aerates the water.

The tanks

The tanks are of normal construction with angle-iron frames and glass fronts, backs, and bases. The one unusual feature is that the ends are of Perspex. Perspex has advantages over glass in that the hole for the connecting units is more easily made and its greater resilience allows for small expansions and contractions due to temperature changes.

The glazing cement is made by mixing, as dry components (proportions by weight), 3 parts litharge, 3 parts silver sand, 3 parts of plaster of paris, and 1 part powdered resin, and then adding enough linseed oil to make it of puttylike consistency. It takes a few days to harden.

This cement is inexpensive but not altogether satisfactory as it tends to become rather brittle in time. In normal use it is quite sound, but careless handling or jolting of tanks might cause it to crack and cause a leak.

As is well known, iron frames need very careful and thorough painting to prevent corrosion. Normal oil-bound paint has been used here, but renovation has been necessary every year or two. Epoxy-resin paints would be well worth trying and are likely to resist corrosion much better.

The dimensions of the tanks and the position and bore of the inlet pipes are of some importance in relation to the maintenance of an adequate rate of water flow. The tanks here are 3 feet long, 8 or 10 inches wide, and 12 inches tall. They contain water to a depth of 3 to 10 inches (see "Tidal conditions" below). The cross-sectional area of the tanks is kept small, as this, combined with the capacity of the pump, largely determines the rate of flow of water (but see note on swirling in the next section).

Tank connecting units

The pipes connecting one tank to the next, and those connecting the 'first' and 'last' tanks to the pump are made of black Alkathene. They are modified from units made commercially for plumbing purposes. The details are shown in figure 5. These units were designed so that a single tank may be removed easily from the series for repair or maintenance. A siphon made of the same material is kept in reserve and is used to maintain the circulation should it be necessary to remove a tank. The first and last tanks are provided with a 'bend' (fig. 4) to which are bolted the adapters for receiving the water from, and conducting the water to, the pump. In all cases watertight seals are obtained by adding a film of vaseline (while the units are dry).

The connecting units are fitted as near to the bottoms of the tanks as possible.

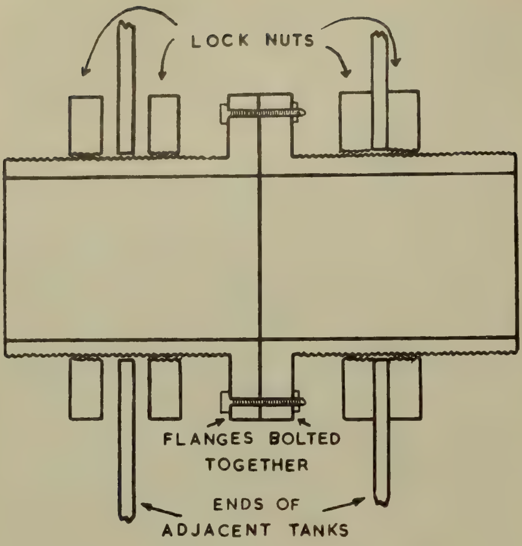

FIGURE 5.—Tank-connecting units.

Swirling of the water is to be encouraged, but increasing the speed of entry of water with a smaller bore pipe (to get more swirling) results in an increase in the head of water and reduces the efficiency of the pump, so that a compromise is necessary; 2-inch bore pipes are used on the aquarium here.

Tidal conditions

A closed circulation system enables tidal conditions to be easily simulated by a sluice gate. A round hole is cut in one of the broad sides of a Perspex box so that it may be fixed to a tank connecting unit. The opposite broad side is cut down so as to be at the level of the water. The sluice gate, also of Perspex, slides in slots against this side of the box. One end of a pivoted arm is attached to the sluice gate, the other end resting on a cam turned by an electric clock motor. Thus the sluice gate rises and falls once every 12 hours; as it rises, the depth of water on the upstream side increases, and therefore decreases on the downstream side; as the gate falls, the two water levels reverse this change until they become equal again.

Tidal conditions are necessary for some intertidal organisms. It has been found that limpets, for example, live indefinitely in these tidal conditions, but were difficult to keep for long without them.

Filtration

For the reasons already stated the water in the aquarium is not filtered. It would be expected that detritus would slowly collect in the tanks, and this does happen. This detritus appears to have no deleterious effects on most organisms, but it does tend to swamp some smaller, sedentary types.

An obvious method of control is to remove it from time to time, but in the absence of knowledge as to the nature of the detritus this practice might upset the chemical balance of the aquarium. A better method is to introduce detritus feeders; hermit crabs have been used successfully.

It is worth mentioning that in spite of the slow accumulation of detritus the water always remains perfectly clear.

Lighting

Fluorescent lighting is provided for some tanks. The amount of lighting, including the time it is switched on, is adjusted so that the photosynthetic organisms (especially diatoms) keep the pH of the water to about 8.1–8.2.

ACKNOWLEDGMENTS

It is a pleasure to record my appreciation of the hospitality of the Director and Staff of the Stazione Zoologica, Naples, where these ideas about marine aquariums were first conceived, and the helpful discussions with many colleagues. I should also like to thank Mr. A. Yeoman, Mr. A. Gall, and Mr. R. A. Fox whose craftsmanship made the aquarium possible.

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A SUBSIDIARY HOT-WATER CIRCUIT IN AN AQUARIUM FED BY AN OPEN CIRCUIT

By L. Barriety

Musée de la Mer, Biarritz, France

Abstract.—An effective method is described for recirculating and filtering heated sea water, in order to alleviate the thermal loss entailed when warmed water is wasted.

One of the disadvantages of supplying an aquarium with sea water using an open circuit is the lack of flexibility from the thermal point of view. The temperature at which the water is received in such an installation is closely related to the temperature of the sea water at the point where it is pumped, and if for any reason—for instance, maintenance of tropical fish or of organisms from polar regions—it is necessary to heat or cool the water, this conditioned water will be drained off almost entirely, without any practical possibility of recovery.

But in these temperate regions it frequently occurs that subtropical animals may be caught from the sea, and if one desires to keep them alive for several years it becomes necessary to supply them with heat during the winter. If they are small, and if the tank where they live is rather small, one may be content to heat the water by any means (the most frequently used means is an immersion heater) and to let the warmed water go down the drain, since the weak output does not involve a very considerable expenditure of heat. Things are entirely different in the case of large animals requiring tanks that are sometimes tens of cubic meters in size. In such a case, the recovery of the water after it has passed through the tanks affords a substantial saving of heat and is therefore necessary. This procedure is used in the turtle (*Caretta caretta*) tank of the Musée de la Mer at Biarritz, which has a volume of 18 cubic meters.

To maintain sufficient limpidity in the tank, it is necessary to ensure a more or less total renewal of the water every 24 hours, and the daily consumption is close to 15 cubic meters. On the other hand, to provide good living conditions for the turtles, the water temperature must be maintained at 17° to 18° C. In midwinter the water comes from the reserve tanks at 10° to 11° C. and is heated in a heat exchanger to 18° C. before it is introduced into the exhibit tank, which it leaves at a temperature of 17° C. It is this water that is drained off, with a consequent loss of the magnitude of 75 therms; this represents, for just one tank, a very considerable waste.

Accordingly, we have considered it appropriate to alleviate that loss as much as possible by recovering the water and reusing it after filtration. Consequently, for this turtle tank, a separate circuit was installed at the outlet of the exhibit tank *A* (fig. 1) which includes a filter with two compartments, a heat exchanger, and a rotary pump which accelerates the circulation. The most interesting element of this

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This paper was prepared in French; it was translated into English by a commercial translating service.

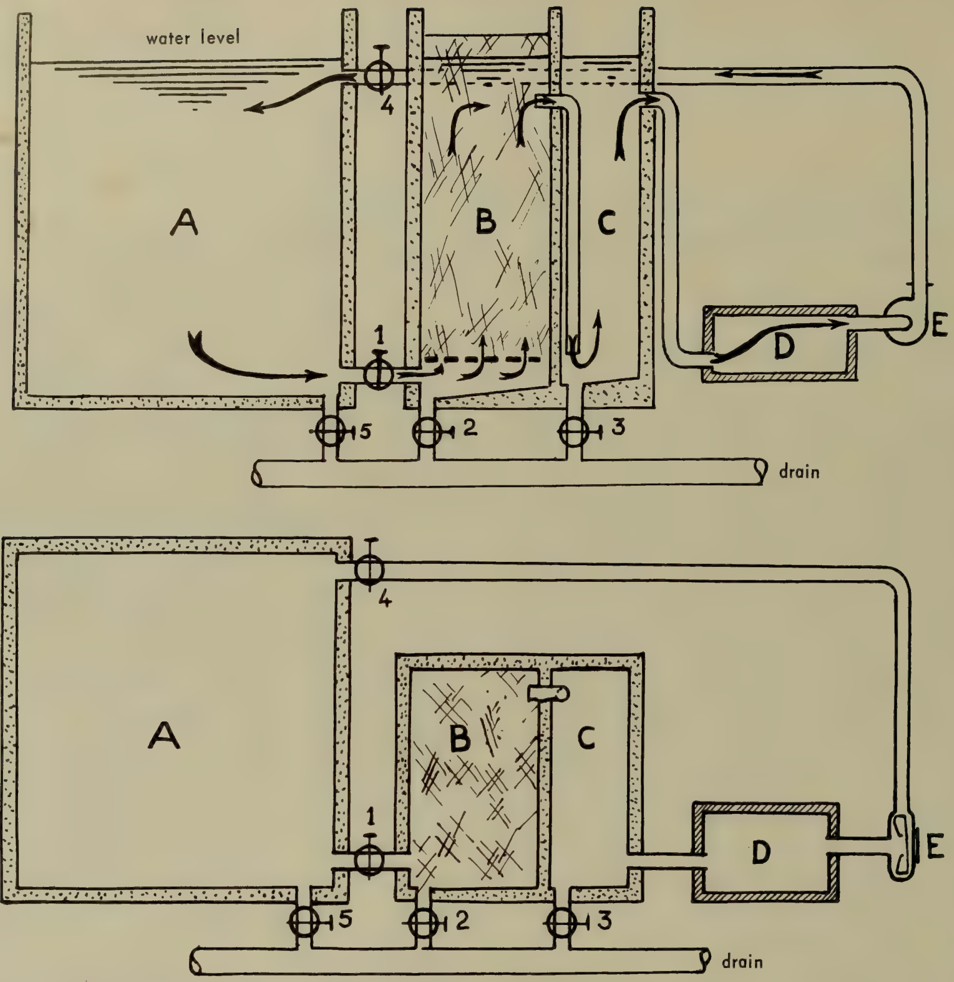

FIGURE 1.—Filtering system—side view above, top view below: *A*, concrete tank; *B*, filter chamber; *C*, storage compartment; *D*, heat exchanger; *E*, centrifugal pump. *1*, *2*, *3*, *4*, and *5* are gate valves.

device is the filter. It consists essentially of a rectangular basin of reinforced concrete from which the flow may be interrupted by means of a gate valve (1). The dirty water arrives from the tank in the lower compartment of the filter (*B′*); the abrupt loss of speed of the water, which flows from the pipe into a much larger vessel, causes a rapid deposition of the largest impurities. The bottom is at a 10-percent incline, with a gate valve (*2*) at its lowest point; this permits easy evacuation of the slush formed. From this lower compartment the water passes through a perforated slab and reaches the filter proper (*b*) which consists first of a layer of oyster shells designed to fix the nitrites, and then of beds of gravel of gradually decreasing size ranging from 5–7 cm. to 1–1.5 cm. The water in the upper part of this filter is sufficiently transparent to be reused in the exhibition tank.

The water is caught once more by a tube which conveys it into a last storage compartment (*C*) where it may still lose, by gravity, a few elements that might have been able to pass through the filtering beds. The lower part of this component of the filter may be cleaned by opening a gate valve (*3*), releasing for drainage a few liters of water from the bottom. Finally, the water will be caught by a pipe system above the last compartment of the filter, and will traverse a heat exchanger (*D*) of 18 kw. where it will be reheated to 18° C., the temperature desired, and finally a centrifugal pump (*E*) which has an output of 2 m.^{3}/h. and is driven by a small 1-kw. electric motor. Even though the pump has a small output, it has enough power for the requirement (about 15 m.^{3}/day); moreover, it does not operate continuously, because the closing and opening of the electric circuit are controlled by an hourly break switch.

The filtering gravel beds are very easy to clean. The gate valve (*2*) is opened, and the pressure is sufficient to direct a jet of water to the upper part of the tank. Some 10 minutes suffice for cleaning the gravel almost completely. It is left to drip off for a few minutes, then the system may be put into operation.

It is unnecessary to add that the installation operates only during the winter. The open-circuit feeding will be reestablished as soon as the pump water reaches a temperature of 16° to 17° C.

It should be understood that the method explained here is only a makeshift device; still, its results are acceptable, and it provides a considerable saving of thermal energy.

EXPERIMENTAL SEA-WATER SYSTEMS FOR REARING FISH LARVAE

By J. E. Shelbourne, *Fishery Research Biologist*

Ministry of Agriculture, Fisheries, and Food Fisheries Laboratory, Lowestoft, Suffolk, England

Abstract.—Marine fish are not easy to rear in aquariums, but encouraging results have been achieved for the plaice (*Pleuronectes platessa*) in closed circulation at Lowestoft. Two temperature-controlled sea-water systems are described, both using illuminated green algae for metabolite regulation, and one having a measure of bacterial control by ultraviolet light. Survival is better in open circulation where metabolite accumulations are less severe. Two further hatchery systems are outlined; one delivering a continuous flow of filtered and temperature-adjusted sea water for local experimental use, and the other for conducting long-term experiments on chemical factors affecting egg and larval survival.

U.S. Fisheries Commissioner Spencer F. Baird (1880) pioneered the first attempt to rehabilitate a depleted sea fishery by large-scale artificial propagation in marine hatcheries. His efforts were influenced by the discovery of G. O. Sars (1866) that a cod egg could be artificially fertilized in much the same way as a freshwater fish egg. The American example stimulated European interest; hatcheries were soon erected in Norway and Britain, but after several decades of operational use, with no solid returns, hatchery practice fell into disrepute. Technical development became stunted by the inability of pioneers to rear sea fish in large numbers beyond their tender early stages. Production was measured in terms of eggs and early larvae liberated into the sea, rather than tough fish with reasonable chances of further survival.

Even in those early days, certain species had been reared through the postlarval phase, including the herring (Meyer, 1880), the cod (Rognerud, 1887), the plaice (Dannevig, 1897), the sole (Fabre-Domergue and Bietrix, 1905) and the turbot (Anthony, 1910), but all on an experimental scale. Mass production in quantities sufficient to influence the yield of a fishery still remains a fish culturist's dream. Work towards this end began at Lowestoft in 1957, the main aim being to develop a small-scale technique which could guarantee a consistent annual target production of metamorphosed plaice (*Pleuronectes platessa*) from sea-spawned eggs. By 1960, up to 10-percent survival had been achieved in closed circulation; this was increased to 33 percent during 1961 in open circulation at Port Erin, Isle of Man, giving survivor densities of 166 fish per square foot of tank bottom.

So far, our technique has developed in an empirical fashion—making mistakes and learning to avoid them. Now that a satisfactory procedure has been worked out, it will be possible to start a more systematic study of factors affecting larval survival, with a reasonable expectation of positive results. This information will help us to streamline our technique and to approach the problem of mass production with economy of effort. Complete technical details and results will begin to appear in the Journal du Conseil Permanent International pour l'Exploration de la Mer during 1962. This paper deals only with the design and operation of our closed and open sea-water circulations.

CLOSED CIRCULATIONS AT LOWESTOFT

The 50-gallon system

In the absence of running sea water at Lowestoft, we were obliged to start fish-rearing studies in static conditions and closed circulations. It soon became evident that small static tanks were unsuitable for survival beyond the early larval stage. By 1957, a closed circulation with limited physicochemical control of water conditions had been designed. It incorporated a fundamental principle of tropical-aquarium technique—the use of photosynthesizing plants to control the CO_2 concentration and stabilize the pH, as well as to remove some end products of protein metabolism and to add oxygen.

The pH can be regulated by chemical means—the addition of lime, for instance (Cooper, 1932), or sodium bicarbonate (Breder and Smith, 1932)—but these methods involve changes in the balance of salts. The CO_2 may also be removed by vigorous aeration (Downing, 1958); cod larvae, however, are known to be harmed by oxygen supersaturation (Henly, 1952). Nitrogenous end products of metabolism cannot be controlled by purely chemical or physical means without affecting the survival of fish in circuit.

The design of our first circulation is shown in figure 1. It has three main components: (*a*) a 30-gallon covered reservoir, (*b*) a covered, temperature-controlled water bath housing two moulded-glass tanks (incubators) each of 8 gallons' operational capacity, and (*c*) a polythene header tank containing illuminated fronds of *Enteromorpha intestinalis* attached to stones. A small centrifugal pump under the control of a mercury float switch intermittently transfers water from the reservoir to the header. The pump volute is best constructed from stainless steel or high-density polythene. The header tank can contain any sessile green alga able to withstand lengthy immersion in water of high salinity. Fronds and substrate must be thoroughly washed to remove mud and debris before use. Tungsten lighting can be used, but a fluorescent source is preferable because it produces less heat. With flexible switching arrangements and a little practice, we found it possible to adjust the light intensity manually, giving a stable pH of 8.1 for days at a time.

Sea water flows by gravity from the header into the incubators at a controlled rate after passing through a nylon mesh screen to hold back plant debris. In circulations of this size, a drip feed of 4 liters per hour per incubator is a practical rate of flow for pelagic eggs and larvae at temperatures up to 10° C. The optimum irrigation rate may differ from species to species; clumped demersal eggs are likely to benefit from a rapid flow. Slow rates of flow can be adjusted by controlling the number of drops falling per unit of time past a "window" (perspex tube) fitted into the inlet system, whilst periodic variations in dropping rate are minimized by setting the header float switch to operate within narrow changes of water level.

Incubator design is a very important factor. Plaice and most other pelagic marine fish larvae are delicate creatures, not adapted to live in constant contact with surfaces. Tanks must therefore be simply constructed, with smooth internal surfaces and no unnecessary inclusions. Two closely apposed surfaces can act as

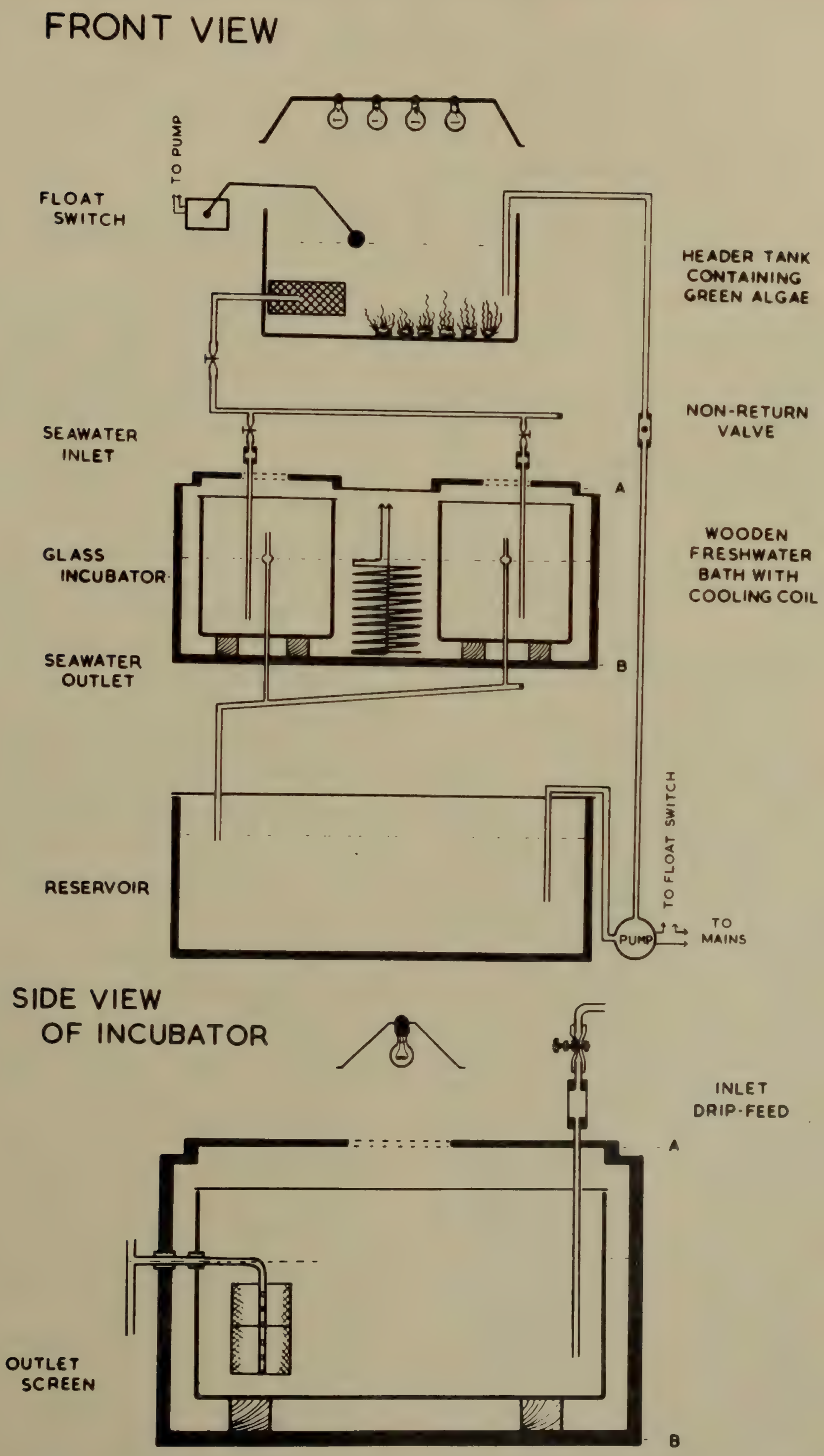

FIGURE 1.—Closed sea-water circulation for rearing plaice: 50-gallon capacity.

a lethal trap; crevices in tank walls are a particular menace—larvae swim into them and seem unable to back out. One incubator inclusion is necessary, however, and that is some sort of screen to prevent eggs, larvae, and larval food from being flushed away. The outlet system shown in figure 1 consists of a submerged vertical polythene pipe, corked at its lower end and perforated along part of its length. The perforations are covered by 155-meshes-per-inch nylon bag, held at a distance by three polythene disks surrounding the pipe. This screen is connected to a horizontal overflow tube passing through a watertight seal in the incubator wall. The nylon mesh is fine enough to retain *Artemia salina* nauplii, a convenient and reliable larval food organism. Plaice larvae are visual feeders; tanks require overhead illumination at water surface intensities around 500 lux during "first feeding." Later on, dimmer lighting helps to depress larval activity in overcrowded conditions. It is also important to black out the walls and bottoms of feeding tanks, since food organisms are more easily seen and captured by early feeders against a dark background.

To regulate water temperature in warm surroundings, incubators are immersed in a fresh-water bath cooled by a copper coil linked to a thermostatically controlled domestic refrigerator unit. The fresh water can be slowly circulated around the incubators by hard aeration. Vertical temperature gradients are inevitable in water-cooled tanks at high ambient temperatures and slow irrigation rates; the agitation required to break them down is detrimental to delicate fish larvae. Controlled air temperature systems are more efficient than water baths. A slow salinity increase occurs in any closed circulation, owing to evaporation. This can be offset by frequent routine additions of distilled water to the reservoir. Copper distillation is not recommended.

In retrospect, the following improvements could be made to the apparatus as described:

1. A substantial increase in reservoir volume.
2. Water-bath cooling replaced by air-temperature control.
3. Automatic pH adjustment, by linking a pH meter control unit to a variable light source above the header.
4. Automatic salinity adjustment, by coupling a salinometer and control to a solenoid valve regulating the input of distilled water into the reservoir.
5. A glass-wool filter inserted at the point where the common outlet pipe discharges into the reservoir.
6. Bacterial control in the reservoir by means of a low-power ultraviolet unit (see later), and in the incubators by occasional dosing with antibiotic mixtures.

Results were encouraging, even without the suggested improvements. They demonstrated that a closed circulation could be used to rear plaice from the egg stage through and beyond metamorphosis—a period of 3 to 4 months—at a minimum ratio of 1 survivor per 2.5 liters of sea water.

The 3,500-gallon system

Our expanded closed circulation (fig. 2), built in 1959, has two main components; a sunken concrete reservoir (15 by 10 by 4 feet) and an adjacent brick hatchery (22 by 13 by 8 feet). The reservoir contains roughly 2,500 gallons of offshore seawater. *Enteromorpha* is once again used to control metabolites, being submerged at a depth of 6 inches, on large trays consisting of polythene film sewn to a wooden framework with no metal fastenings. The alga receives illumination at night from fluorescent tubes arranged in banks of four above the trays. Each bank hangs by a nylon rope and pulley (permitting vertical adjustment) from a stout timber gallows spanning the tank.

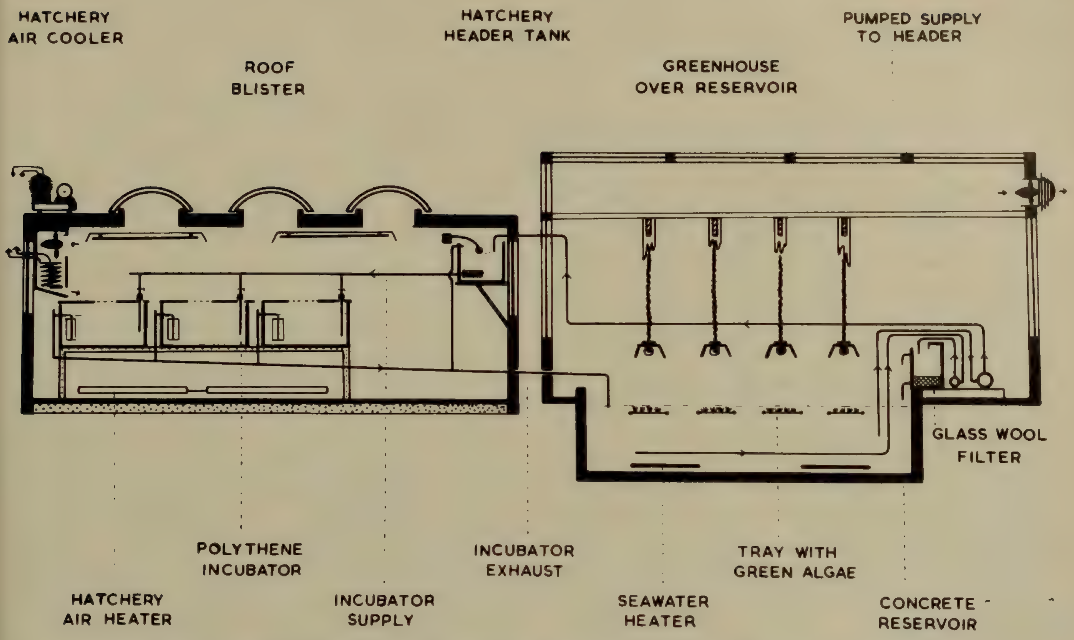

FIGURE 2.—Closed sea-water circulation for rearing plaice: 3,500-gallon capacity.

A greenhouse covers the reservoir, so it receives natural lighting during the day.

Low-wattage immersion heating panels. embedded in epoxy resin and controlled by floating thermostats, counteract excessive cooling of reservoir water during cold weather. For 2 years there were no safeguards against rapidly rising water temperatures in spring and early summer. A separate cooling circulation combined with filtration and bacterial control was installed in 1961 (fig. 3). Sea water is pumped at a fast rate from the far end of the reservoir into three plywood boxes irradiated with ultraviolet light. After treatment, water overflows out of the boxes, through glass-wool filters, into three 100-gallon asbestos-cement tanks, each containing two refrigeration coils made up from 50-foot lengths of ¾-inch steel tubing. All six coils are connected to a 3-horsepower motor and compressor controlled by a thermostat in the reservoir. Water overflows from the cooling units back into the main tank.

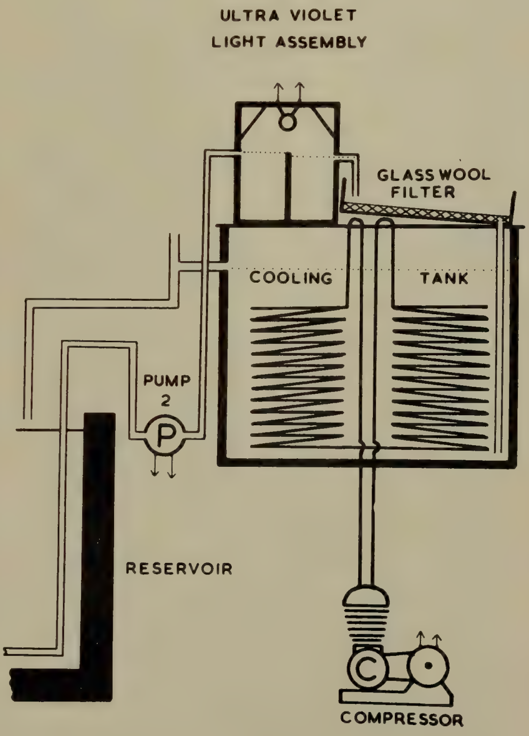

FIGURE 3.—Reservoir cooling circulation combined with filtration and ultraviolet irradiation.

A small electric pump operated by a mercury float switch transfers sea water from the reservoir to a header tank in the hatchery. From there it is gravity-fed to nine 5- by 2- by 2-foot black polythene rearing tanks on metal stands. Although much bigger than the glass incubators shown in figure 1, their design is essentially the same. Water returns to the reservoir along a common exhaust tube. The inside of the hatchery is painted white; glass "blisters" in the hatchery roof provide overhead illumination during the day, augmented by four 80-watt fluorescent tubes. Hatchery air temperature is stabilized by heaters in cold weather and two small air coolers in summer.

Metal surfaces in contact with sea water must be kept to a minimum; among the cheaper metals only stainless steel is safe. Metallic fittings are best coated with epoxy resin to prevent corrosion and the release of harmful ions; mild-steel coils treated in this manner can be used for cooling sea water by direct immersion. Filters are necessary to check undesirable phytoplankton blooms in the reservoir and to reduce the surfaces available for bacterial attachment. There is some doubt about the efficiency of our bactericidal arrangements in 1961; the matter is discussed in the next section.

In retrospect, it was a mistake to separate hatchery and reservoir. Both components should be housed in the same insulated building equipped with air-temperature control. Sunken concrete reservoirs can be replaced by fiberglass tanks above floor level; direct water-temperature control systems then become superfluous. Natural lighting may be an advantage for efficient algal photosynthesis, and could be provided by double-glazed panels in the roof. Fluorescent lighting at night is in this case preferable to tungsten, on grounds of heat production, which can be further minimized by siting lamp-control gear outside the building.

Bacterial control in closed circulation

Oppenheimer (1955) demonstrated the possible value of bacterial control by antibiotics in marine fish hatcheries. Walne (1958) also used antibiotics to increase the survival rates of oyster larvae in experimental tanks. Bacterial populations are usually higher inshore than offshore (ZoBell, 1946). The plaice and many other sea fishes spawn in virtually oceanic waters with a low bacterial count. In the rich organic environment of a closed circulation the bacteriological problem must be greatly magnified. Even at low incubation temperatures less than 6° C., plaice egg shells become covered with epiphytic bacteria, a condition seldom occurring in the sea. The effect of shell contaminants on final survival to metamorphosis has not yet been systematically assessed; a start is to be made during the spring of 1962.

Wood (1961) has studied the use of ultraviolet light for bacterial control in seawater circulations, and our system is based on his design. Reservoir sea water flows at a total rate of 300 gallons an hour through three plywood boxes lined with epoxy resin (fig. 4). Each lid contains two 15-watt low-pressure ultraviolet tubes backed by an aluminium reflector. Water enters the box at a low level and passes over a longitudinal plywood weir before overflowing into the filters. We were unable to test the bactericidal efficiency of the system before installation; an opportunity to do so occurred later on, when the plaice-rearing experiments were finished.

A supply of 450 gallons of turbid estuary water in a concrete tank was continuously pumped through two of our ultraviolet boxes at a fast rate of 520 gallons per hour. Water samples were withdrawn at frequent intervals, and bacterial

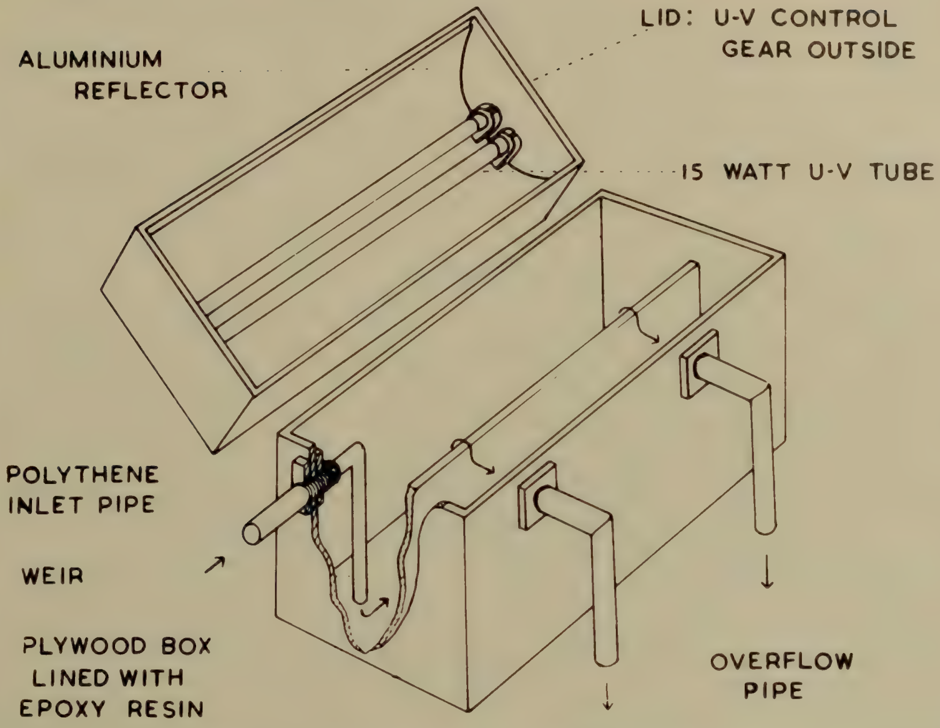

Figure 4.—Design of ultraviolet bactericidal unit.

populations were assessed by mixing 1-ml. volumes with ZoBell's 2216 medium using the standard pour-plate method. Duplicate bacterial counts were also derived from the dropping technique of Miles and Misra (1938). Even at a flow of 260 gallons per hour per box, water leaving the outlets was virtually sterile after treatment. Within 6 hours, the originally high bacterial counts characteristic of inshore water had dropped to a level similar to that in the open sea (fig. 5). The treatment was continued overnight, and sampling resumed the following morning. By this time the water in the reservoir was perfectly clear and the filters were caked with estuarine debris. The filter bypass was then closed and the whole flow was directed through the ultraviolet boxes alone. Figure 5 demonstrates (*a*) the bacterial efficiency of these units at a high rate of flow and (*b*) the recontaminating influence of dirty filters in a bypass circuit.

The hourly flow in our 1961 plaice-rearing circuit was only one-tenth of the reservoir volume. Thus the bactericidal potential of the system was not fully exploited. In addition, the filters were incorrectly sited—they should be placed before and not behind the source of ultraviolet radiation. Given rapid water turnover, reservoir bacteria can undoubtedly be controlled by direct irradiation. However, the method is not easily applied to bacterial control in actual rearing tanks, where periodic dosing with antibiotics may be necessary, at least during the egg phase.

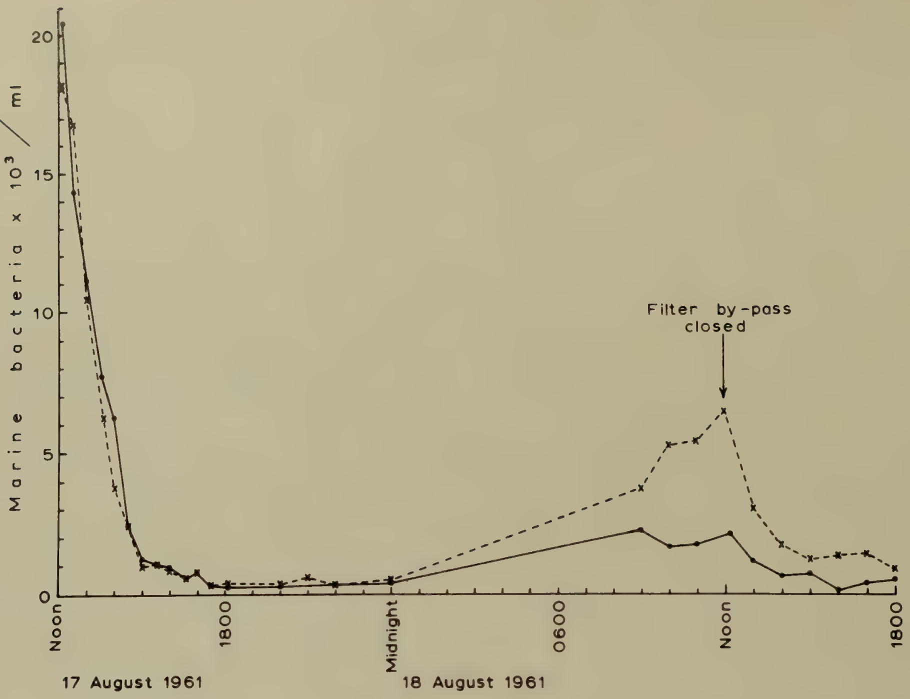

FIGURE 5.—Survival curve of marine bacteria irradiated in closed circulation. Solid line indicates pour plate (1-ml. water samples in ZoBell's 2216 medium). Dashed line indicates Miles and Misra method (1/50-ml. drops on ZoBell's medium). Plates incubated at 25° C. for 24 hours. Mean water temperature 18° C.

OPEN CIRCULATION AT PORT ERIN, ISLE OF MAN

Metabolites are continually removed in open circulation, and survivals of plaice larvae are correspondingly higher. A mean survival of 33 percent was achieved in the control tanks of a salinity experiment at Port Erin during 1961. This is several times greater than the expected result in closed circulation at Lowestoft, using the same basic tank design and handling technique.

Hatchery sea water is usually of the inshore variety; certain controls are still necessary. For instance bacterial control is needed either in the main reservoirs or locally at the point of flow into experimental tanks. Filtration is also important in open circulation since suspended particles provide a "foothold" for sessile bacteria. The problem of temperature control still persists with diurnal fluctuations in the coastal environment; salinity similarly varies according to rainfall and local runoff.

Local filtration and temperature control

A simple apparatus to continuously filter incoming hatchery sea water, and to lower its temperature before delivery into experimental tanks, is shown in figure 6. It consists of a 10-gallon polythene carboy fitted with a high-level overflow pipe and a low-level delivery tube leading into an insulated 40-gallon asbestos-cement header tank. The tank contains a resin-coated steel cooling coil coupled to a ⅓-horsepower refrigerator unit outside the hatchery, controlled by a sensitive thermo-

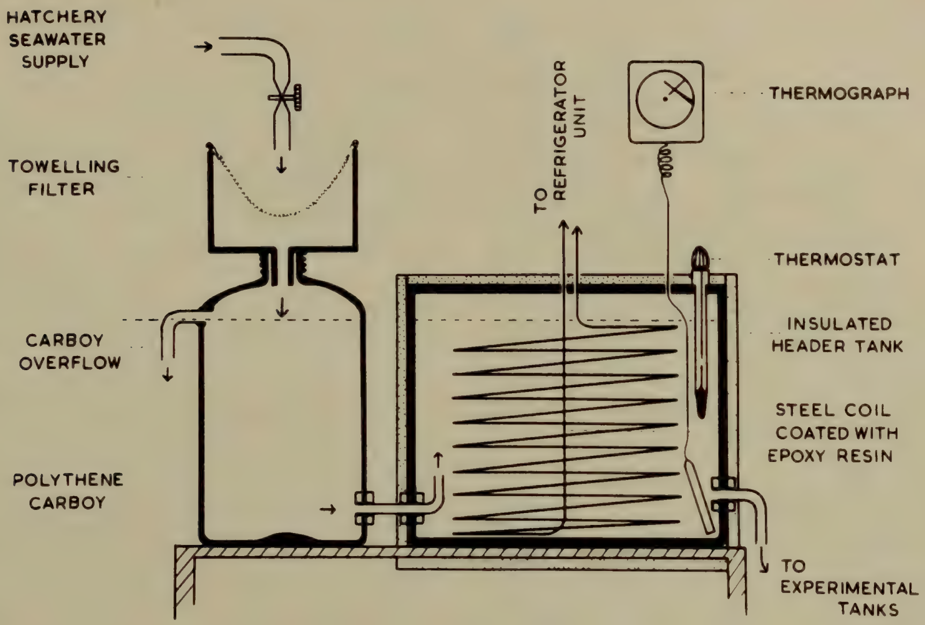

FIGURE 6.—Open-circulation apparatus providing local filtration and temperature control.

stat in the header. Hatchery sea water passes into the carboy through two layers of fine-weave toweling, at a rate slightly in excess of experimental requirements. The filtered overflow runs to waste. Temperature-adjusted water leaving the header is continually replaced by filtered water from the carboy. Some mechanical stirring may be necessary in the control tank; no float switches or ball cocks are required. If the temperature of the incoming seawater is lower than required, an aquarium heater replaces the cooling coil. Both heater and cooling coil can be used to counter one another when the temperature of the incoming supply is very variable. In practice, the unit delivered 30 gallons per hour of filtered sea water at 7° C., with the main hatchery supply standing at 10° C.

The horizontal stippled strip below the supporting platform of the header tank is a sheet of expanded polystyrene insulation board, stuck on the underside. It represents a not-too-necessary refinement if the platform is of thick timber, and could be eliminated if one so chooses. The vertical and topside stippling similarly represents the insulating sheath of the tank.

Port Erin sea water is relatively clear, but in turbid conditions a finer filter system will be needed, with an increased filtration surface. A small-wattage bactericidal unit can easily be interposed between the filter and the constant-level carboy. The pipeline to rearing tanks, and the tanks themselves, must be insulated if hatchery air temperature is uncontrolled. An apparatus of this sort ran for 3 months during 1961 with no attention other than a daily change of filter.

A variable salinity apparatus in open circulation

Most salinity experiments on marine fish eggs and larvae have been confined to testing the effect of extremes on short-term survival in static conditions. We are more interested in the long-term effect of slight differences in salinity. An open circulation is essential, since accumulating metabolites may mask a salinity effect. Figures 7 and 8 illustrate an experimental unit successfully used in 1961.

A measured amount of hatchery sea water is filtered through toweling into a leached asbestos-cement reservoir (100-gallon capacity) each morning, and a calculated volume of distilled water is added to give a desired lower salinity. After thorough mixing, this water is continuously transferred by a small airlift pump into a polythene header tank above the reservoir, at a rate slightly in excess of incubator requirements. A delivery manifold emerging from the front lower face of the header, conducts water at an equal and steady rate of 4 liters per hour into each of three 2- by 1- by 1-foot glass incubators. The header overflow is channelled back into the reservoir. This arrangement provides a constant head to facilitate delivery control.

The three glass incubators have the same design as that illustrated in figure 1. Their sides are shrouded in close-fitting jackets of black polythene film. The inlets deliver their flow below the water surface; each perforated outlet pipe has a vertical extension, also below the surface, screened with a fine nylon mesh bag held at a distance from the pipe by three polythene disks. The incubators sit side by side in a black fiberglass tank through which sea water slowly circulates from the main hatchery supply. This water bath is calculated to limit the effect, if any, of diurnal variations in hatchery air temperature. The outlet (strictly overflow) pipes of the inner glass tanks are extended through the side of the fiberglass water bath, to discharge into a common drain gutter. The bath has a plywood cover, treated underneath with epoxy resin, and painted on

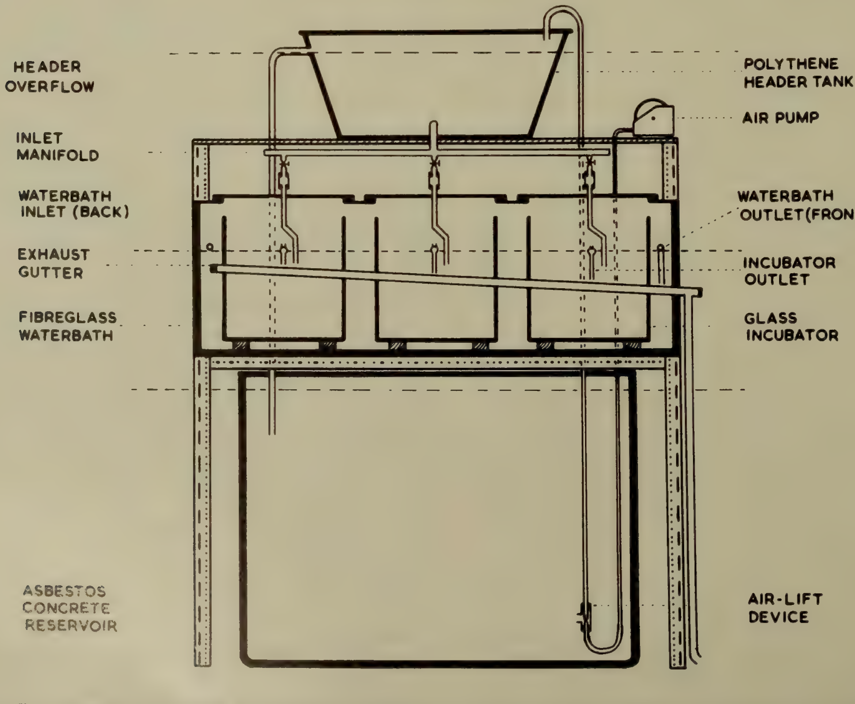

Figure 7.—Open-circulation apparatus designed to test long-term effect of salinity variation on the survival of plaice larvae: front view.

top. Hinged plywood flaps over large rectangular holes in the cover give access to the inner tanks. These flaps are provided with central slits covered by translucent polythene film, for admitting light. A prefabricated steel framework carries all components other than the reservoir, which stands on the floor, partly hidden under the water bath.

The volume of salinity-adjusted sea water made up each day is always more than that required for 24 hours' irrigation. Residual water is measured the following morning with a dipstick, and the amount of distilled water in the mixture calculated. Measured volumes of hatchery sea water and distilled water are then added to provide the next 24 hours' supply.

By this method it is possible to maintain a continuous flow of sea water adjusted to a salinity below that of hatchery supply, for long periods of time. Adjustments the other way can be made by adding suitable mixtures of salts. The experimental tanks are buffered against air temperature variation only. Stricter temperature standards would require a hatchery air-conditioning plant; alternatively, the water bath could be made part of an auxiliary closed fresh-water circulation with immersion heating or cooling. These triple tank units are virtually self-contained and can be used in replicate to test any long-term chemical effect on larval survival, without running the risks inherent in a static system.

All the closed and open circulations described in this paper have been successfully used to rear plaice larvae. Technical details such as optimum temperatures, light, salinity, pH, flow rate, and diet will vary from species to species, but these basic designs are recommended as a starting point in the experimental study of hatchery requirements for all marine fish with pelagic eggs.

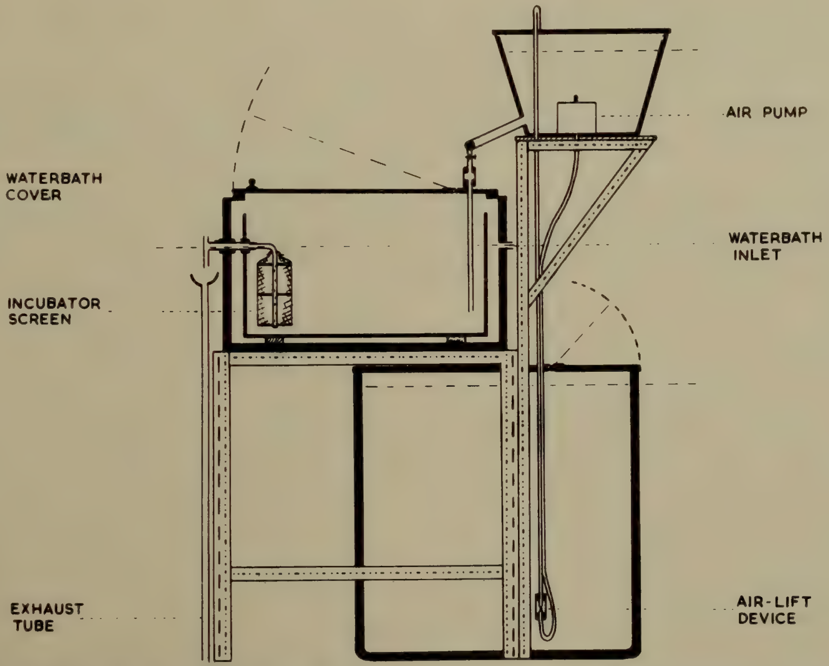

FIGURE 8.—Open-circulation apparatus designed to test long-term effect of salinity variation on the survival of plaice larvae: side view.

SUMMARY

Hatcheries were built in America and Europe at the turn of the nineteenth century to rehabilitate depleted sea fisheries by artificial propagation. They fell into disuse mainly as a result of ill-developed techniques. Marine fish are difficult to rear beyond the tender larval stages, even on a small scale, but a plaice-rearing technique has been empirically evolved at Lowestoft giving up to 10-percent survival from egg to metamorphosis in closed circulation. Two temperature-controlled sea-water systems are described, both using illuminated green algae for metabolite regulation, and one having some degree of bacterial control by ultraviolet light.

Survival is better in open circulation, where the metabolite problem is minimized. A system for providing a continuous flow of filtered and temperature-adjusted sea water is described, together with an apparatus for conducting long-term experiments on chemical factors affecting larval survival.

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A WORKING BIBLIOGRAPHY ON REARING LARVAL MARINE FISHES IN THE LABORATORY

By James W. Atz, Curator

New York Aquarium, New York Zoological Society, Brooklyn, N.Y.

Abstract.—The literature available on bibliographic resources on rearing larval marine fishes in laboratory aquariums is reviewed critically; the list of references papers of special interest are designated. A list of marine teleost fishes reared in captivity is included.

It has been said that God made an animal to solve every problem in physiology and that the principal task of the experimenter is to find the species most suited to his needs. Although this statement contains a greater element of faith than even those who would never use it impiously might realize, the number of different laboratory animals available to the experimental biologist has continued to increase over the years along with the increase in number and variety of experiments.

As experimental animals, however, fishes have lagged behind the members of the other vertebrate classes, and only a minuscule fraction of the estimated 15 to 40 thousand species has found its way into the laboratory. The relatively remote phylogenetic position of the fishes and a few of their specific attributes, such as smallness of cells, have made them unpopular, but the principal obstacle to a more widespread use of fishes as laboratory animals is their limited ability to maintain homeostasis, which makes them delicate experimental subjects and establishes quite rigid requirements for their successful maintenance in captivity. This has been particularly true of the marine species, which are generally much more stenokous than those inhabiting fresh or brackish waters. Moreover, the limitations imposed by the inherent stenoky of marine fishes are aggravated by the difficulty of establishing a stable environment in salt-water aquariums.

The same factors have impeded the domestication of fishes; no marine species has yet been domesticated, principally because no salt-water fish can be bred generation after generation in captivity (Atz and Pickford, 1959). Domestication is not necessarily a prerequisite for even extensive use in the laboratory—witness those laboratory favorites, the true frogs (*Rana* spp.) and the mummichog (*Fundulus heteroclitus*)—but all domesticated animals are, of course, available to the experimentalist as, for example, are rainbow trout (*Salmo gairdnerii*), carp (*Cyprinus carpio*), guppy (*Poecilia reticulata*), and Siamese fighting fish (*Betta splendens*). There would be an immediately realized monetary advantage in spawning and rearing the milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) for pondfish culture, but the potential value of being able to study under controlled conditions all phases of the life histories of the marine fishes that will soon provide the bulk of man's animal protein food is inestimable.

The author's present address is American Museum of Natural History, New York, N.Y.

About 50 years ago, artificial fertilization and incubation of the eggs of marine food fishes were carried out on a large scale in order to replenish defining natural stocks (e.g. see papers presented at the Fourth International Fishery Congress, Washington, 1908, which were published in 1910 in vol. 28 of the Bulletin of the U.S. Bureau of Fisheries). A critical look at the biostatistics involved (e.g. by Breder, 1922) demonstrated the uselessness of the procedure, which was eventually abandoned. Little systematic effort was made to rear the fish, since they were released or planted shortly after hatching, and Morris (1956) found that few data helpful for laboratory culture had been recorded.

Hertling (1932) and Morris (1956) give accounts of some of the early attempts to rear marine fishes from the egg. Much of the difficulty encountered was referred to the so-called critical period, when the larva, having used up its yolk supply, becomes dependent on other, external sources of food. While recognizing the radical nature of this vital change in larval nutrition, Morris makes it clear that in some species this is not an especially critical period, in the sense that no greater mortality is suffered at the time of the change. Marr (1956) reviews the evidence for a critical period in nature and concludes that it is not well demonstrated. Well taken is the point made by Morris that until the sensitivity of any given species has been determined, "the situation can be considered critical from the time of fertilization until organogenesis is completed."

Because of the rigid requirements of the eggs and larvae of marine fishes, data delimiting their tolerance of environmental factors and establishing optima should be especially useful. The series of papers by Bishai (1960a, 1960b, 1960c, 1961a, 1961b), supplemented by Holliday and Blaxter (1960) and Blaxter (1960), provides a convenient and comprehensive summary of this autecological approach. A point to be remembered, however, is that the limits of tolerance of eggs and larvae for conditions in captivity are often less than those exhibited in the wild, probably because of the generally suboptimal artificial environment. Moreover, not all of the environmental factors affecting fish larvae in nature have been studied. For example, attention has recently been directed to possible lethal effects of light (Perlmutter, 1961), and this element should be given consideration by the experimentalist.

The extreme sensitivity of fish larvae in general, and of those of marine species in particular, to substances in the water surrounding them is well recognized. Containers made of chemically inert materials such as glass and certain plastics must be used, preferably those that have never been in contact with formaldehyde, cleaning solution, and the like (Morris, 1956; Costello et al., 1957). It is now feasible to construct a small yet efficient circulatory system entirely of nonmetallic materials—a vital piece of laboratory equipment that remained an impractical ideal for many years. A satisfactory formula for artificial sea water in which fish larvae have thrived has yet to be developed, while MacGinitie (1947) has pointed out that natural sea water "rots on standing, and the resulting chemical and pH changes are lethal, particularly to larval forms." These changes are undoubtedly the result of the tremendous multiplication of bacteria in stored sea water; they may be controlled with antibiotics (Oppenheimer, 1955; Marshall and Orr, 1958; Rustad, 1960; Shelbourne, 1963a) or ultraviolet radiation (Wood, 1961; Herald et al., 1962), but cleanliness, aeration, filtration, and storage in the dark remain the principal means of maintaining sea water in a satisfactory condition. Since eggs and larvae are sensitive to metabolic wastes—ammonia and perhaps other substances—crowding is to be avoided.

One of the knottiest problems in raising marine fishes concerns their nutrition. Morris (1955, 1956), Qasim (1959), and Hirano (1963) experimented with various foods and determined that one of the most successful was newly hatched brine shrimp (*Artemia salina*). A general account of this phyllopod crustacean and its use may be found in Dempster (1953). The San Francisco Aquarium Society and the California Academy of Sciences are sponsoring the publication of a comprehensive bibliography on *Artemia*, which should be published by the Society during 1964. The compendium edited by Needham et al. (1937) contains a section devoted to saltwater aquariums as well as instructions on rearing several kinds of marine invertebrates.

MARINE TELEOST FISHES REARED IN CAPTIVITY

A summary of marine teleost fishes which have been successfully reared in captivity from the egg is given in the following list:

Family Clupeidae:
- Atlantic herring, *Clupea harengus harengus* Linnaeus. [Kotthaus, 1939; Soleim, 1950; Dannevig and Dannevig, 1950; Dannevig and Hansen, 1952; Blaxter and Hempel, 1961a, 1961b; Bishai, 1961c; Blaxter, 1962.]

Family Gadidae:
- Atlantic cod, *Gadus morhua* Linnaeus. [Burd and Jones, 1948; Dannevig and Dannevig, 1950; Dannevig and Hansen, 1952; Dannevig, 1963; Wise, 1963.]

Family Gasterosteidae:
- Threespine stickleback, *Gasterosteus aculeatus* Linnaeus. [Leiner, 1934; Heuts, 1947.]

Family Aulorhynchidae:
- Tube-snout, *Aulorhynchus flavidus* Gill. [Morris, 1956.]

Family Syngnathidae:
- Spotted seahorse, *Hippocampus erectus* Perry. [Herald and Rakowicz, 1951; Simpson, 1957.]
- Dwarf seahorse, *Hippocampus zosterae* Jordan and Gilbert. [Strawn, 1954, 1958.]

Family Sparidae:
- Black porgy, *Milio macrocephalus* (Basilewsky). [Kasahara et al., 1960.]

Family Embiotocidae:
- Barred surfperch, *Amphistichus argenteus* Agassiz. [Triplett, 1960.]
- Shiner perch, *Cymatogaster aggregata* Gibbons. [Triplett, 1960.]

Family Scombridae:
- Black skipjack, *Euthynnus lineatus* Kishinouye. [Clemens, 1956.]

Family Cottidae:
- Bald sculpin, *Clinocottus recalvus* (Greeley). [Morris, 1956.]
- Fluffy sculpin, *Oligocottus snyderi* Greeley. [Morris, 1956.]

Family Blenniidae:
- Butterfly blenny, *Blennius ocellaris* Linnaeus. [Garstang, 1900.]
- Shanny, *Blennius pholis* Linnaeus. [Qasim, 1955, 1959.]

Family Pholidae:
- Rock gunnel, *Pholis gunnellus* (Linnaeus). [Qasim, 1955, 1959.]

Family Atherinidae:
- Atlantic silverside, *Menidia menidia* (Linnaeus). [Rubinoff, 1958.]
- Topsmelt, *Atherinops affinis* (Ayres). [McHugh and Walker, 1948.]
- Jacksmelt, *Atherinopsis californiensis* Girard. [Morris, 1956.]
- California grunion, *Leuresthes tenuis* (Ayres). [McHugh and Walker, 1948; Morris, 1956.]

Family Bothidae:
- Turbot, *Scophthalmus rhombus* (Linnaeus). [Anthony, 1910.]

Family Pleuronectidae:
- Plaice, *Pleuronectes platessa* Linnaeus. [Rollefsen, 1939; Dannevig and Dannevig, 1950; Dannevig and Hansen, 1952; Shelbourne, 1956, 1963a, 1963b; Rustad, 1960; Shelbourne, Riley and Thacker, 1963; Riley and Thacker, 1963.]
- Lemon sole, *Microstomus kitt* (Walbaum). [Dannevig and Dannevig, 1950.]

Family Soleidae:
- Sole, *Solea solea* (Linnaeus). [Dannevig and Dannevig, 1950.]

Family Tetraodontidae:
    Puffer, *Fugu pardalis* (Timminck and Schlegel). [Shōjima, 1957.]
Family Batrachoididae:
    Oyster toadfish, *Opsanus tau* (Linnaeus). [Straughan, 1957; Tracy, 1959; Dovel, 1960.]

From this list it can be seen that less than 30 species of marine teleosts have ever been reared in captivity from the egg. The few successes achieved can be attributed, in general, to two causes. The first concerns those economically important species on which it has been worthwhile to spend a good deal of time and effort (Atlantic herring, Atlantic cod, and the flatfishes), and the second those species with well-developed hatchlings or with special means of parental care, either of which might be considered to preadapt them to successful reproduction in the laboratory (sticklebacks, tube-snouts, seahorses, atherinids, surfperches, and toadfishes). We were surprised not to find any papers that describe the raising of *Fundulus heteroclitus* or some other marine cyprinodont in captivity, because members of the family Cyprinodontidae would seem to be eminently suitable for this kind of laboratory life. The eggs of the mummichog are easy to obtain and hatch (Gabriel, 1944; Costello et al., 1957), and the young have been raised to adulthood with simple equipment and relatively little effort (Alfred Perlmutter, New York University, personal communication). We have also seen sheepshead minnows (*Cyprinodon variegatus*) hatch from eggs laid in one of our exhibition aquariums and grow to near maturity with no special care whatsoever.

The work of Triplett (1960) indicates the possibility of culturing the young of the viviparous surfperches throughout the period of embryonic development, which would normally be spent within the ovary—just as that of Trinkaus and Drake (1952) had previously shown for the well-known poeciliid, the guppy. Perhaps similar techniques could be applied to the other types of livebearing marine fishes, i.e., the Clinidae (kelpfishes), Zoarcidae (eelpouts), and Brotulidae (brotulas). Those scorpionfishes and rockfishes of the family Scorpaenidae that bring forth living young are truly ovoviviparous, however, and their newborn young are no further developed than those of the scorpaenids that lay eggs; indeed, Morris (1956) was unable to carry newly born *Sebastodes goodei* to metamorphosis. A priori, it would seem that the best chance for success would be with a form lying somewhere between the extremes of true ovoviviparity and viviparity, since the latter would involve placentalike structures and almost complete dependence of offspring on mother for nutrition, for which it might be as difficult to find an adequate substitute as it has been in the mammals. The elasmobranchs run a similar gamut from ovoviviparity to viviparity, but no attempts to grow in vitro the embryos of sharks, dogfishes, rays, sawfishes, or guitarfishes seem to have been recorded. On the other hand, the large eggs of the oviparous sharks and skates have proved excellent experimental subjects (Vivien, 1941, 1954).

Whether the eggs and young of the marine fishes that practice oral incubation would be especially suited for laboratory culture is a pertinent question. Shaw and Aronson (1954) and Shaw (1957) have shown that the eggs and young of one of the fresh-water, mouth-breeding cichlids can be cultured outside the parent's mouth. There appears to be no reason why similar techniques would not work with the sea catfishes (family Ariidae), although there might be a problem in feeding the young fish, which are known to ingest food during the latter part of the 2-month period spent in the father's mouth. In the cardinalfishes (family Apogonidae) and jaw-fishes (family Opisthognathidae), however, the spawn is held together in a single mass, and in the former group at least, the young are not incubated and hatch at an unadvanced stage.

The following bibliography makes no claim to completeness, but it will serve to introduce the investigator to a scattered literature. Those items indicated by an asterisk contain information of general interest and should be consulted no matter what species is to be investigated.

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ESTUARINE WATER SYSTEMS AT SOLOMONS, MARYLAND

By David G. Cargo

Natural Resources Institute of the University of Maryland, Solomons, Md.

Abstract.—The novel features of two salt-water systems at Solomons, Md., are discussed. Details of the straight-through rising-pipe supply system, progressing cavity pumps, constant-head trough distribution system, and tank arrangements are included. Drawings of these and other associated details are set forth along with a brief evaluation of the two systems and the maintenance schedule employed. A list of sources of equipment is included.

The sea-water systems at the Chesapeake Biological Laboratory might be more aptly termed estuarine-water systems. At this laboratory, approximately halfway between ocean and fresh water, the problems and advantages of each situation are present in addition to circumstances peculiar to a brackish-water environment. Long-term average values of daily salinity show a seasonal range from 9.9 to 16.9 parts per thousand. Similar temperature values range from 2.7° to 27.8° C.

Our primary aim is to provide an estuarine habitat of limited scope which can be observed and, to some extent, controlled so that laboratory experiments can be performed on a continuous basis. While bringing this habitat into the laboratory, every effort was made to avoid changing the chemical or physical character of the water. We envisioned such facilities for use in live storage or holding of experimental animals, in hatching and raising marine organisms, in studying ecological responses of various organisms to controlled and varying conditions, and in short-term educational display. Our success in providing systems capable of answering all of these needs and aims is yet to be determined.

The sea-water systems in current use at Solomons are of recent origin. Since experienced professional advice was unavailable to us, much of our design embodies ideas and suggestions contributed by scientists at this and at other similar marine laboratories. There are two complete and separate sea-water systems. The first system has been in use about 3 years and is installed at a research storage building and, although simple, is proving an extremely useful and reliable arrangement. This system will be referred to as the "wharf system." The second more recent and much more sophisticated system is located at the main research building and has been in operation for about a year and a half. It is still too early to provide an overall evaluation of its potentialities.

There are several features embodied in both of these systems which may be of interest. These contribute in various ways to make the system work better. In some cases, a simplification resulted; in others, the modifications made the system more

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complex. Although final evaluation is not yet possible, we feel that the general result of our efforts has been encouraging.

I am indebted to many persons of this and other institutions who have assisted us in various ways during the designing and installation of these systems. Particular credit is due Dr. L. E. Cronin, Director of the Natural Resources Institute, for his efforts and perseverance in planning for this invaluable and necessary tool for research.

THE WHARF SYSTEM

The wharf system is a simple, straight-through system utilizing plastic piping and a hard-rubber centrifugal pump. Flowing at 80 gallons per minute, the water, after passing through the pump, flows up a continuously rising supply pipe provided with petcocks at frequent intervals. This rising supply was designed to insure that the pipe remains full despite the level of use. This feature has been found to be very efficient (fig. 1).

The rather rapid flow through the system forced us to provide the terminus with a throttling-type valve to provide increased pressure when needed. This valve was required because, when a petcock was opened, the rapid flow aspirated air into the system instead of allowing water to flow from the petcock. This valve induces sufficient back pressure to overcome this aspiratory tendency. We feel that this valve is a necessary component.

We have found that this system remains virtually free of internal fouling of all types except bacterial. During the warm parts of the year, external fouling requires a cleaning of the foot valve and strainer every 10 days to 2 weeks. During the colder periods of the year, this schedule may be extended considerably. Our treatment involves raising the intake head and scraping and scrubbing the external fouling. We then drain the line and flush it thoroughly with hot (130° to 150° F.) fresh water. This hot water is allowed to remain in the line until it has cooled (about 2 hours). The fresh water is then pumped out after the head has been returned to the normal pumping position in the water. This schedule has been markedly satisfactory throughout the 3 years of operation. Occasionally, the motor of the pump is greased sparingly. The pump has required no maintenance or lubrication since installation.

The main features of this system are installed in a special room which has been designed for the handling of samples—washing, sorting, holding, etc. Incorporated in the construction of the building are underfloor drains, and future expansion of the utility is provided by pipe carriers extended to the outside of the walls so that this same system can be used to supply outside tanks if needed at some future date.

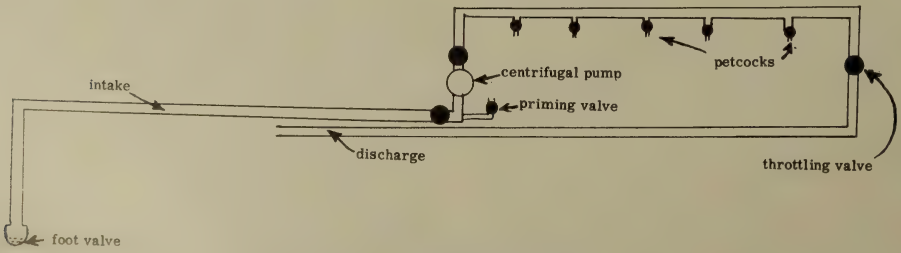

Figure 1.—Wharf system.

THE MAIN SYSTEM

The main laboratory research building, about an eighth of a mile from the wharf site, is now supplied with sea water by a dual supply and pump system. The pumps are Moyno progressing cavity units manufactured by Robbins and Meyers. These were chosen in an effort to provide an adequate volume of water with a minimum of change in pressure and turbulence. No metal comes in contact with the inflowing water, and the pumps were specially modified to this end. Each is powered by a 3-horsepower, 230-volt motor, 1750 rpm. The double V-belt drive reduces the pump speed to about 800 rpm.

The stators of these pumps are rubber. The rotors, usually made of stainless steel, are molded of polyurethane. The pumps are further modified by the fabrication of several other parts of nylon instead of stainless steel and by coatings of polyvinyl chloride. The pumps act to entrap successive small volumes of water and push them through the pump with a minimum of turbulence, agitation, or pulsation. Since the entire system is guarded against toxicity from metal and is capable of providing a large volume of water at a relatively low pressure, we expect to be able to conduct a wider range of experiments under conditions which compare favorably with those in the estuary.

Our distribution system in the aquarium room consists of a boxlike trough, suspended from the ceiling joists on brass rods. This trough (figs. 3 and 4) is made of cypress and is coated on the inside with an epoxy-base clear sealer. The divider strip, running longitudinally in the trough, allows a 2-way flow in the trough. The primary aim of this trough is to combine a

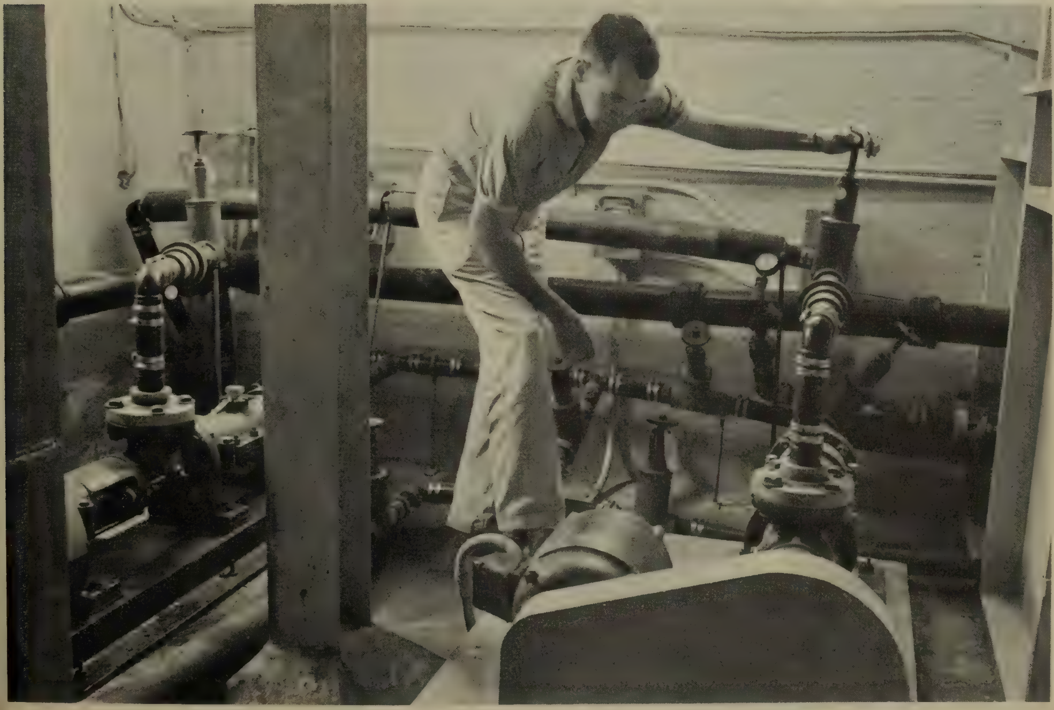

Figure 2.—Pumproom detail.

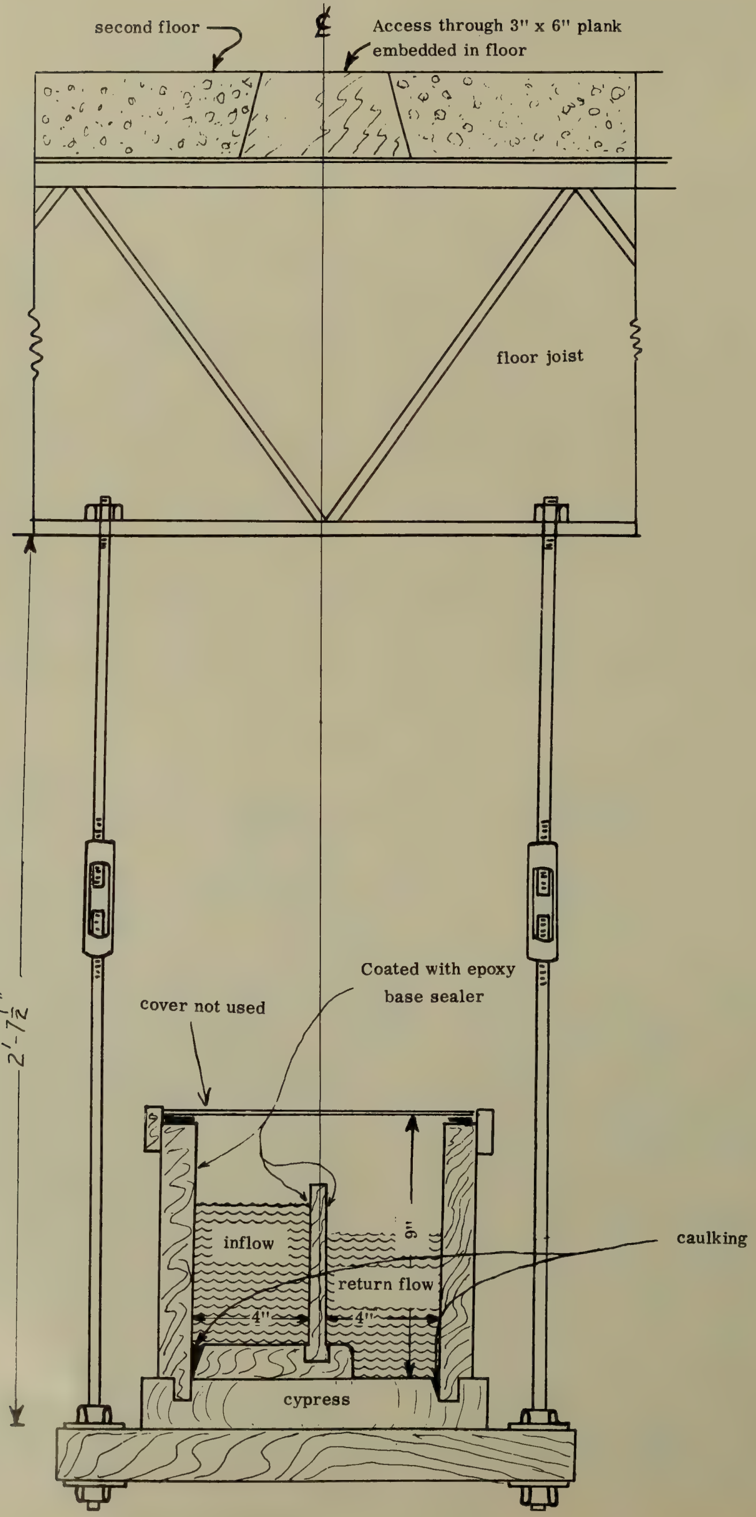

FIGURE 3.—Trough detail.

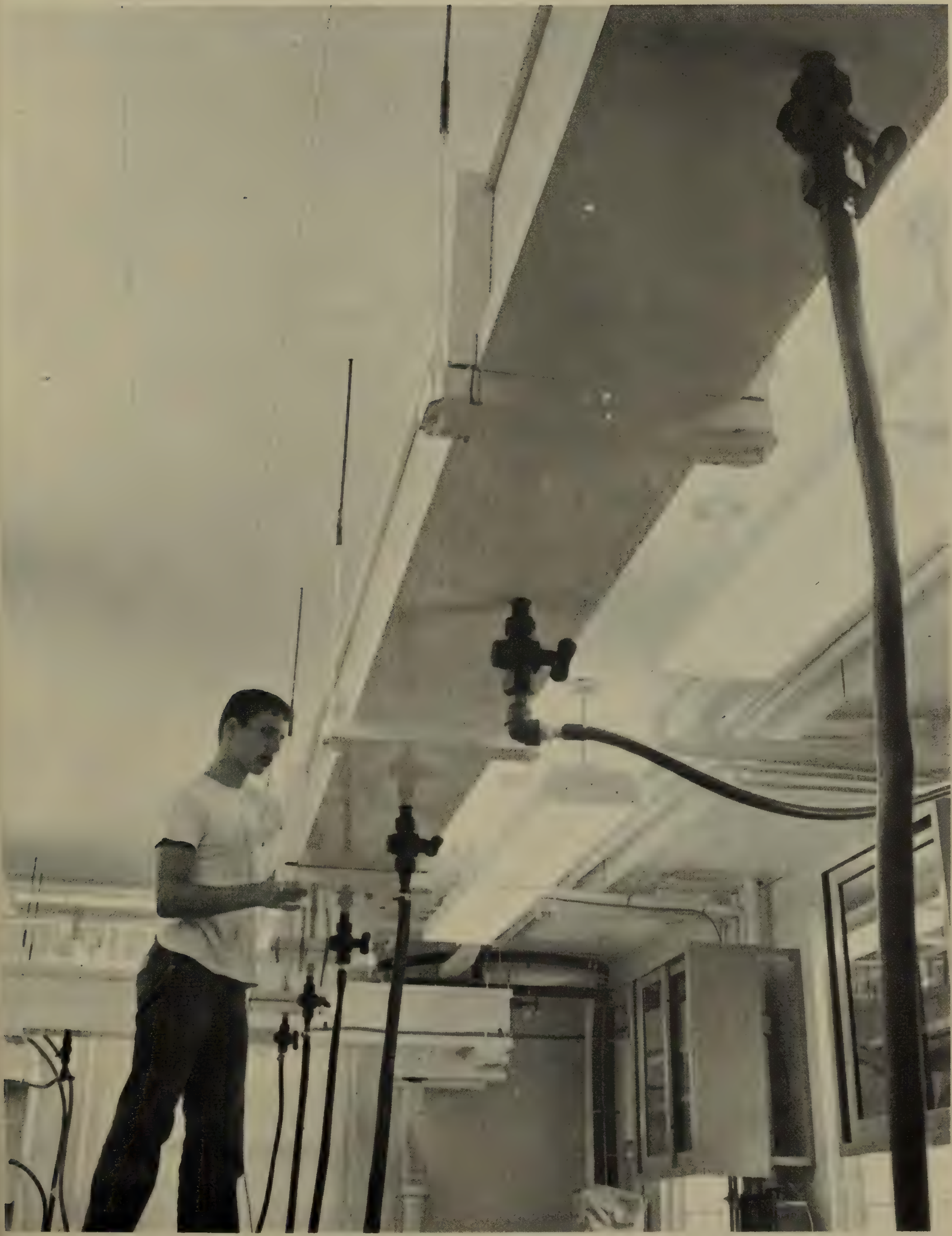

FIGURE 4.—View of overhead trough showing petcocks.

simple distribution system with a constant-head supply so that the control of the various taps is not as critical as it would be if it were a closed, pressure-type system. For distribution to the tanks, an undersized hole is bored in the bottom of the trough, a tap is run through the hole, and a hard-rubber petcock is threaded into the hole. Our original design included weirs at several places (fig. 5) which were designed to maintain a level in the trough. Actually, only the weir at the discharge box is required; the remainder have been removed.

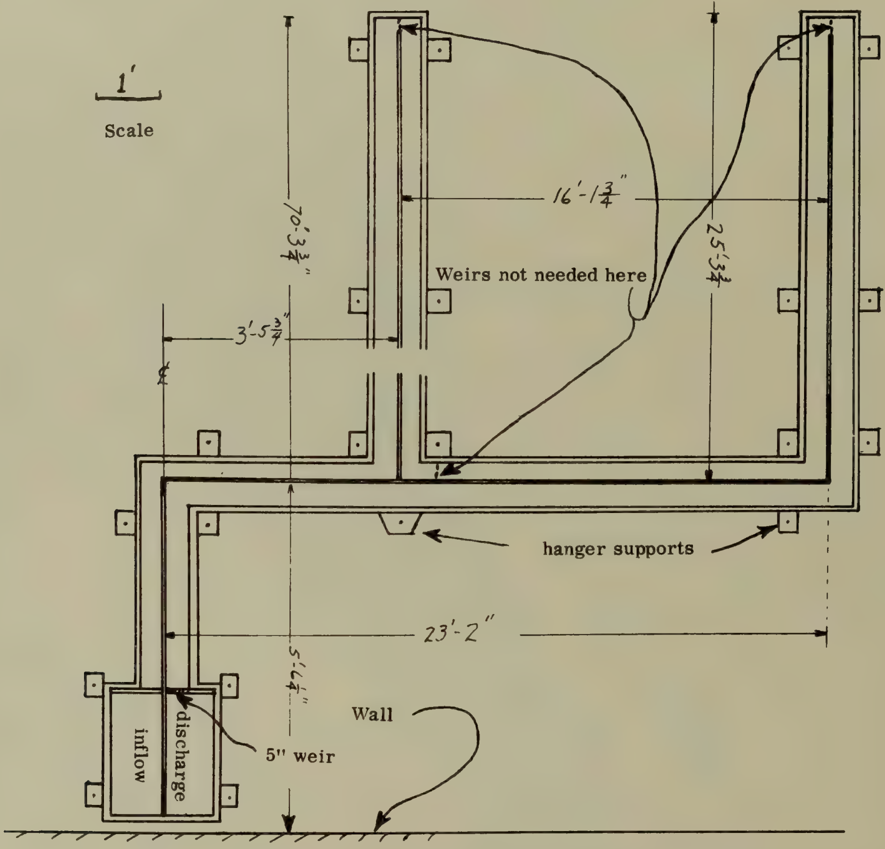

Figure 5.—Plan of distribution trough.

In use, the trough has required a regular scraping and removal of the accumulated fouling. Fortunately, most of this fouling occurs in the first one-quarter of the path that the incoming water travels. Access to the trough from the second floor of the research building is provided by a wooden plank embedded in the concrete floor. Thus, the boring of an access hole is an easy matter.

The aquarium tanks, shown in some detail in figures 6 and 7, have proved very successful. The stainless-steel bolts can be covered with a synthetic-rubber compound. The condensation on the inner walls has not proved bothersome; however, some leakage has been experienced so that the groove for carrying off condensation has seen considerable use. The problem of adequately sealing the glass to the concrete has not been completely solved, but we have not considered the slight leakage worth the effort of resealing these places.

Several other features of the tanks merit

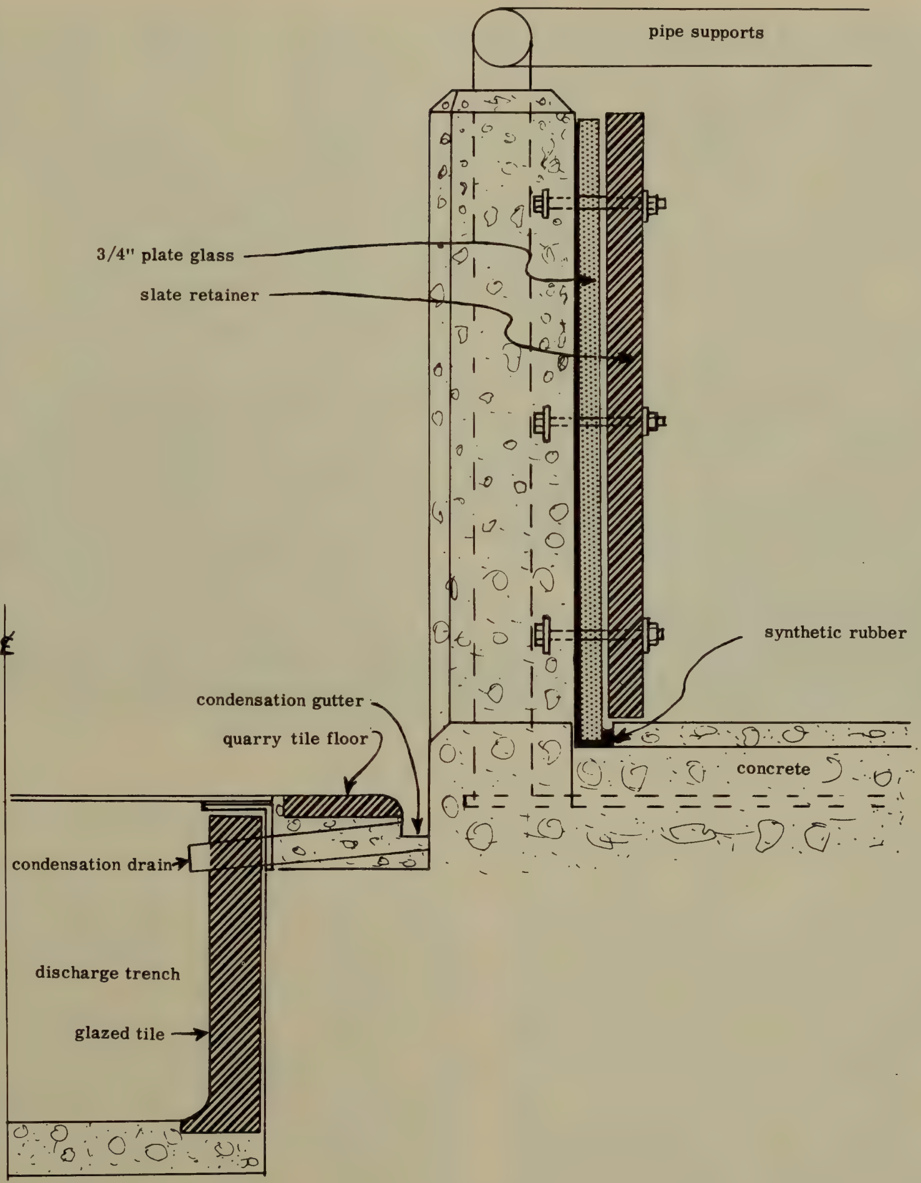

Figure 6.—Detail of aquarium construction.

special attention. The heavy pipe supports (fig. 7) are of double-strength pipe, fabricated into one welded framework and galvanized. These supports are designed to serve as a base for other aquariums or tanks, cooling and heating equipment, and monitoring and controlling components. They are heavy enough to support a volume of water comparable to the tank below. Although not obvious in the photograph, the glass partitions between the tanks are held in place with replaceable packing and are removable. This feature permits us to expand the size and utility of these tanks. We have employed 3-inch standpipes of PVC plastic in these tanks which allows us to adjust, through the use of plastic pipe plugs, the height of the

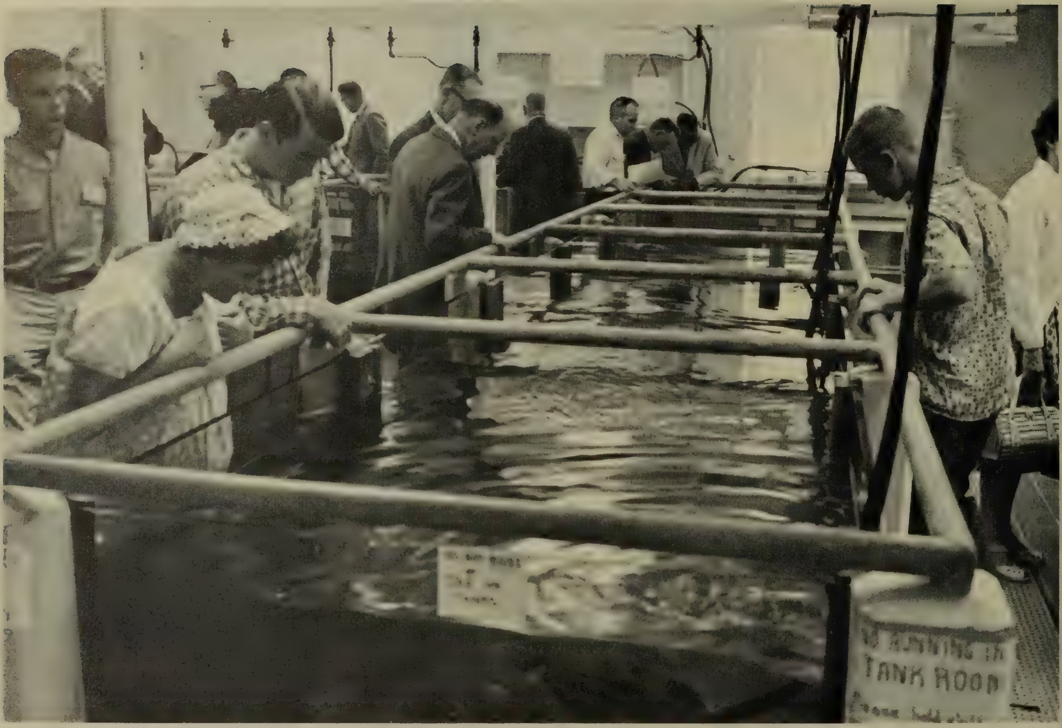

FIGURE 7.—General view of aquariums.

water in the tanks. These plugs are threaded into holes tapped into the sides of the standpipes.

The entire pumping system (fig. 8) is flushed regularly with fresh water held in a 500-gallon tank and coupled into the system. This water is not heated. Some minor fouling occurs inside the foot valve but has not proved troublesome. This same backwash water is used for priming the pumps when necessary. Naturally, this fresh water is not allowed to enter the supply trough when purging the lines.

A feature common to both systems is a strainer which is necessary to avoid the clogging of the intake by jellyfish and ctenophores. After trying several designs, we found that a large polyethylene wastebasket pierced by numerous ¼-inch holes and attached to a wooden disk clamped to the intake pipe answers this need nicely. By releasing two bolts (we used plastic bolts), the unit can be disassembled and scrubbed. The wooden disk was not painted but is still in good condition after 18 months' use. In general, this screen arrangement has proved very efficient and satisfactory.

EVALUATION AND REMARKS

The wharf system has provided us with an extremely reliable source of running water for a period of almost 3 years. Initial problems with priming and the strainers were quickly resolved as our familiarity with the equipment increased. The entire system was installed by our maintenance staff at the site; installation required no outside assistance.

The main research system has given problems which might be considered commensurate with the complexity of the system. Certain mechanical failures have resulted, principally from unfamiliarity

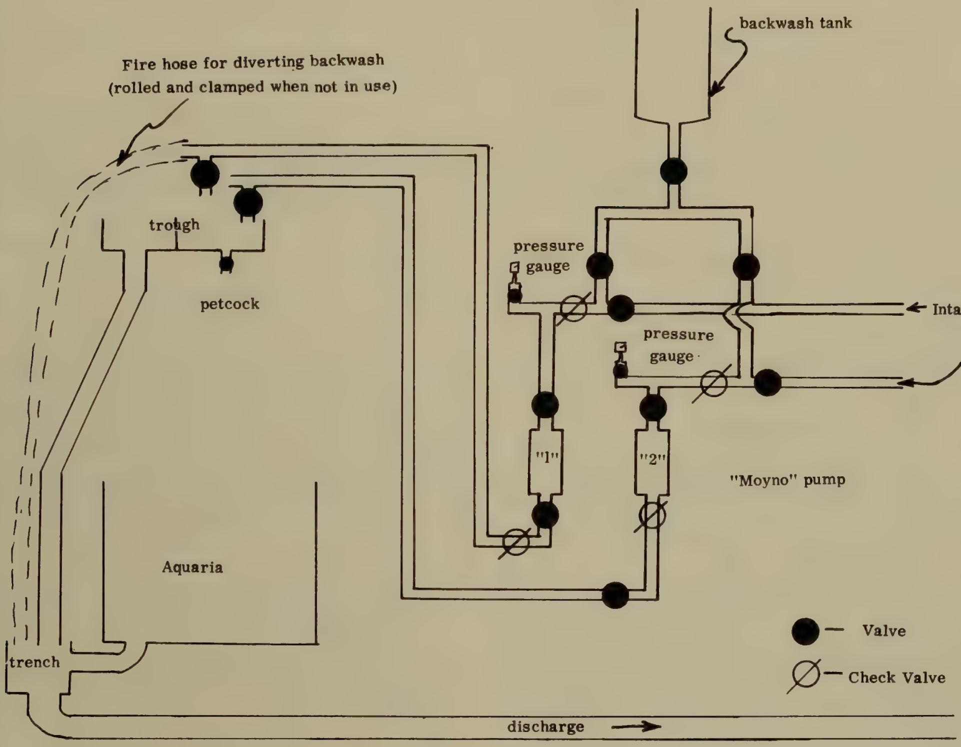

FIGURE 8.—Main system (dual).

with the equipment and a lack of adequate manufacturers' instructions concerning maintenance requirements. It is felt that our present schedule will enable us to operate on a more satisfactory basis.

Our aim of producing water at a low pressure was answered in a most heartening manner when numbers of small medusae of *Chrysaora q. quinquecirrha* were observed swimming in several of the tanks. These were about 1 to 1½ inches in diameter and appeared to be in excellent condition. Since that time, practically all of the local larval forms have passed through the pumps successfully and most have metamorphosed in the trough or tanks. Thus, our present feeling is that these pumps are worth the effort and expense.

We have had several failures due to circumstances which could not be counteracted at the time of their occurrence. It is therefore strongly recommended that a safety switch and relay be made a part of any system so that a stoppage or slowing of the water flow for any reason will shut off the pump, thus protecting the unit from serious damage. Of equal importance is the provision for thermal overload protection for each motor. We recently lost a motor because of a low-voltage condition which caused the field windings to overheat and short-circuit.

The following is a list of suppliers of the components we have used in our system.

Centrifugal pumps: American Hard Rubber Co., Butler, N.J.

Moyno pumps: Robbins & Myers, Springfield, Ohio.

PVC and hard-rubber valves, pipe, fittings: American Hard Rubber Co., Butler, N.J.

PVC and hard-rubber valves, pipe, fittings: Vanton Pipe and Equipment Co., Hillside, N.J.

Polyethylene piping, fittings: American Hard Rubber Co., Butler, N.J.

Polyethylene piping: Sears, Roebuck & Co., Philadelphia, Pa.

Polyethylene piping and fittings: Yardley Plastics, Columbus, Ohio.

Vinyl and polyethylene tubing and containers: Phipps & Bird, Inc., Richmond, Va.

Epoxy-base paint: Pettit Paint Co., Belleville, N.J.

Black asphaltum varnish: Devoe & Raynolds Co., New York, N.Y.

Pressure switches: Barksdale Valves, Los Angeles 58, Calif.

Stainless-steel pipe clamps: Murray Co., Towson, Md.

SUMMARY

The two systems for supplying salt water for the Chesapeake Biological Laboratory at Solomons are essentially metal-free, continuous-running systems. One provides 80 gallons per minute through a centrifugal pump, the other 25 gallons per minute through a progressing cavity pump at a low pressure. The first is a straight-through, positive-pressure unit of simple design. The second is more complicated, with a novel and efficient distribution system.

Both of these systems have their advantages and disadvantages but present experience indicates that an increased efficiency and utility will be realized in the future as our familiarity with these units is improved.

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SEA-WATER SUPPLY IN THE TROPICS

By Warren Wisby

University of Miami Marine Laboratory, Miami, Fla.

Abstract.—The existing sea-water system is described for institutions in need of a moderate supply of sea water. Special methods were devised to eliminate frequent maintenance, save space, accommodate an extremely high ground-water level, and permit continuous operation in spite of tropical storms. Additions and changes for a contemplated enlarged facility are discussed, covering the control of temperature, salinity, pH, dissolved oxygen and CO_2, and turbidity in the incoming water. Methods are given whereby programed variations of any controllable parameter may be maintained.

When, some months ago, a new laboratory was being planned for the Institute of Marine Science of the University of Miami, the existing salt-water pumping system was subjected to intensive scrutiny and examination to determine which of its features were generally deemed worth retaining and which were to be improved or discarded. The experiences of our staff members in various marine laboratories, in this country and abroad, were drawn upon. Conferences were held with experts from various disciplines, including biologists, corrosion engineers, chemists specializing in toxicity studies, and hydraulics engineers. The plans which resulted from this search and evaluation program retained many features of the existing system. In the belief that a description of the existing system would be of value to institutions with moderate sea-water demands, it will be described in the first portion of this paper. The remainder of the paper will describe the contemplated system in detail.

EXISTING SYSTEM

The salt-water supply system now in use in the Agassiz Building of the Institute of Marine Science was installed when the Institute's laboratory facilities were moved to their present location on Virginia Key in 1953, and has been in continuous use since that time. A few relatively minor changes have been made in the system; these were due mainly to an increase in the demands placed upon it by a greatly expanded scientific staff. When, some years later, a second salt-water laboratory was planned, it was decided that the existing pumping and distribution systems had proven their worth, and a duplicate system was installed in that facility, now our Collier Building. These two similar systems have managed to provide the entire supply of salt water for the Institute until the present time when, again because of increasing demands, they were judged inadequate and plans for a new salt-water facility were started. The simplicity of their design recommends them primarily for small installations. However, many of their design features should, and can, be incorporated into more extensive systems.

The Institute of Marine Science is on Virginia Key on the shore of Bear Cut, a narrow strait connecting a shallow part of Biscayne Bay with the Atlantic Ocean. Water conditions in Bear Cut vary from relatively clear water free of suspended materials, on an incoming tide, to rather turbid water with a silicaceous silt load on an outgoing tide. Turbidity increases when winds are high and especially when high winds are combined with spring tides.

The intake for the present salt-water system is fastened to the laboratory pier and consists of a screened foot valve, which is easily removed for cleaning, within a sturdy box which is itself fitted with easily removable, coarse, stainless-steel screening. The foot valve is located about 6 feet under the surface of the water at mean tide, placing it about the same distance over the bottom. The intake line, about 50 feet in length, is composed of 3-inch black polyethylene pipe and leads to a manifold which is connected to the suction side of two Jaeger centrifugal pumps, each of which is powered by a 3-phase, 1-horsepower motor. The pumps have a rated capacity of 50 gallons per minute under these operating conditions and are so valved that either pump can be placed in service. The initial cost of these pumps is remarkably low, and it is therefore feasible to keep standby units on hand. Furthermore, repairs are not costly and are, as a matter of fact, infrequent. This type of pump is commonly used in construction work, and repair facilities should be available in most cities. Repairs have usually consisted in replacement of worn impellers and an occasional volute, neither of which requires a large expenditure of funds or of time since the pump can easily be loaded into an automobile and taken to the repair shop.

The discharge line is also manifolded at the pumps and is also composed of 3-inch black polyethylene pipe, which has been covered with aluminum paint so as to reduce absorption of heat by the water. This line empties into one end of a concrete tank (10-by-10-by-2-feet) situated on the roof of the building. Here the flow is diffused over a maximum cross section and rendered relatively nonturbulent, by means of removable baffles, in order to reduce the rate of linear flow so that most of the settleable materials are removed. The water level is maintained by means of an electric float switch which controls that pump which happens to be in service. The tank is equipped with a hinged cover to exclude sunlight and accompanying algal growth, and a removable overflow drain which leads back to the bay. Removal of the overflow standpipe allows the settled materials to be flushed and the tank to be cleaned.

The secondary, or distribution, system leaves through a screened outlet located a few inches above the tank floor. It is constructed of the same kind of pipe as are the suction and primary discharge lines. Branches of this system travel down each side of the building for final distribution to the water tables and aquariums. In no case does any pipe have a blind, capped end. Rather, the end of each pipe is fitted with a valve and a discharge line which leads back to the bay, so that the accumulation of sediment, with resultant formation of H_2S, can be prevented by frequent flushing of all parts of the system. The same arrangement is provided in the smaller lines which supply the individual aquariums. Also it was found that sedimentation ceased to be a factor in clogging of valves, which are all of plastic (PVC), if the lines which supplied the aquariums, as well as the plastic stopcocks from these lines, were connected to the upper side of the distribution line instead of the bottom, as is usually the case.

The water tables are constructed of fiberglass-covered plywood, are standardized as to size and design, and are easily movable. Each has two levels which are supplied with running sea water, and each level is connected by a drain hose to a gutter which carries the waste back to the bay.

The feature of the distribution system which is considered to be the most important is that nowhere in the entire system is an elbow joint used. Where it is necessary to turn a corner, a four-way fitting is used and the unwanted openings are capped. Thus, any section of pipe can be thoroughly cleaned with a plumber's "snake" merely by removing the cap that leads in a straight line to the proper section. We have found through experience that such cleaning must be part of the regular maintenance schedule owing to the rapid growth of settling organisms in the tropics.

SALT-WATER SYSTEM FOR NEW BUILDING

The sea-water system designed for the new laboratory remains relatively unchanged from this original concept. It will, of course, be much larger and more sophisticated as to controls, but the basic principles have been judged sound and time-tested and will be adhered to. All primary and secondary piping will be of PVC plastic, including the valves, and will be installed in duplicate down to the final distribution level. Provisions will be made to keep unused portions filled with fresh water to destroy any marine organisms which may have settled during their periods of use. Our experience has shown that if this is done bimonthly, the majority of settling organisms will be so small that the calcareous shell will be removed from the walls of the pipe when the organism dies. Provision will also be made for flushing the used portion of the system with a fast flow of sea water daily, thus preventing sediment from accumulating in the piping in use.

The question of filtration was again examined in detail and the conclusion was the same as that arrived at when the existing system was planned—that a settling tank on the roof was most suited to our needs. Our water supply was first subjected to scrutiny. It was found, by drilling test wells, that our underground salt water, although crystal clear, contained so much dissolved H_2S as to render it unsafe for use with small marine organisms, even after treatment. Also, while it might be possible to maintain living fishes in this treated water, whether they could then be considered "normal" in activity and in behaviour is questionable.

Mechanical and biological filters were also discussed and discarded; the former because of the difficulty of cleaning and the frequency with which this would be necessary with our water conditions, and the latter because of the undesirable contribution of additional larvae and eggs to the already present problem. It was also decided that the settling tank should be on the roof, as at present, for several reasons. First, pressure systems, the other alternative, are not satisfactory in the tropics because of the danger of supersaturation of dissolved gases with even slight warming of the water, with resultant trauma to the tissues of the experimental organisms. Second, our ground water is so near the surface as to make digging difficult. Third, a large underground tank would have to be kept filled with water in order to prevent its "floating," especially in the event of storm flooding, and this would make cleaning difficult. Fourth, a ground surface tank would take up valuable space and would necessitate two sets of pumps, one to pump into the tank and one to pump from the tank to the roof of the building, where a gravity tank would be installed.

Tests were again conducted on the rate of settling of the materials suspended in the intake water, and it was found that a retention time of 90 minutes was sufficient for the large majority of our experiments. The tank was therefore designed to provide a retention time of 1½ hours at the maximum pumping rate of 36,000 gallons per hour. It will cover most of the roof of the building and will be shielded from the direct rays of the sun by a permanent cover, which also acts to prevent dilution by rain water.

Provision has been made for cleaning by dividing the tank into two parts. Either part can function as a settling tank while the other is shut down. The settled water will be collected in a sump at the end of the tank for final distribution to the laboratories. This will ensure a constant head of water to the secondary piping system. In many cases this water will require no further filtration. Nevertheless, unit filters will be generally available, to be used or bypassed as desired. The filter units are plastic, with disposable filter elements, and are similar to those successfully used elsewhere. The cost of the elements is relatively small, they can be obtained with various pore sizes, and should last from 2 to 3 days in an average installation before cleaning or replacement is needed.

Many of the problems of a salt-water system have to do with the corrosive nature of the sea water, while others are due to the erosive effects of suspended materials. A search was instigated to find a pump which would resist the effects of both of these factors. The investigation has narrowed down to a centrifugal pump with a special rubber lining. A total of three pumps will be installed in parallel connection. The pumps will be actuated automatically by a demand signal from a float-type switch sensing the water level in each distribution sump. As many pumps will be in operation as are required to maintain the desired head of water.

As in our present sea-water system, all piping will consist of straight runs without bends or elbows. Four-way fittings, or tees will be used at all corners so that a cleaning rod can be inserted easily. It is planned to use unplasticized nontoxic PVC piping both in the primary and in the secondary distribution systems. All valves will be of similar inert materials, either PVC, Teflon, or hard rubber, depending upon their size. Automatic control systems will be provided for regulating the following environmental parameters. (See Addendum on p. 118.)

Temperature

A number of thermostatically controlled, portable units for heating and cooling the settled sea water will be available for individual use.

Salinity

Water conditioning will take place in a mixing chamber before being introduced into the aquariums. Salinity levels will be sensed by means of a conductivity cell, or inductive type cell, and automatic regulation applied to the inflowing water supply. Salinity will be reduced by the metering in of distilled water or of fresh water previously passed through a dechlorinator. Salinity will be increased by spraying raw salt water into an evacuated chamber with subsequent reoxygenation. A salinity control of ± 1 part per thousand can be provided.

pH

Adjustment of pH will take place in a tank before the sea water enters the aquariums. Measuring electrodes will be glass (Beckman) with a calomel reference. The measured values will actuate the control system so that an acid or a base will be metered into the mixing tank at a rate sufficient to maintain the desired pH.

Dissolved oxygen

Measurement and recording of the dissolved oxygen will be accomplished with either Kanwisher or Beckman type electrodes. Oxygen level may be set and maintained at any desired level between O and saturation. Deviations of the measured level from the present level will provide the control signal for adjusting the oxygen level in the incoming water supply. Oxygen will be added to or removed from the water by passage through a column of glass beads into which oxygen or nitrogen is metered.

Dissolved carbon dioxide

Measuring and control systems similar to those provided for oxygen will be supplied. Carbon dioxide will be added to or removed from the sea water by trickling through a glass bead column into which carbon dioxide is metered or CO_2=free air is passed. An adjustment of pH will be made along with the regulation of carbon dioxide.

Turbidity control

Measurement of turbidity will be made by absorption of light as compared to a neutral density filter. Filters of a wide range of optical density may be inserted into the reference optical path of the control system, and turbidity can then be automatically maintained at a level of equal light absorption. Increase of turbidity can be accomplished by metering in a suspension of the material under study or of some natural sediment into the incoming water supply, or it can be decreased by filtering. The type of material to be used will depend on whether visual effects or effects of sedimentation on bottom organisms are being investigated.

System for maintaining programed variations of any controllable parameter

This system will consist of a graphic recorder and controller which will follow a curve previously plotted on the chart paper. Any desired fluctuation of a variable (as a function of time) can be plotted on the chart paper by hand. As the graphic record is played back, the stylus is made to follow the excursions of the plotted line while at the same time producing a control signal which is a function of its lateral displacement. The control signal is used to regulate the environmental parameter so that it follows directly the preplotted graphic record. The time scale may be synchronized with real time if desired. Fluctuations in such variables as temperature, salinity, or light may be provided in a cyclic manner so that tidal or diurnal cycles are simulated. Noncyclic variations may be programed in the same manner.

SUMMARY

The following problems, some of which are peculiar to the tropics, have been dealt with in the design of the new sea-water system:

1. Sedimentation and growth of fouling organisms in the pipes by (a) filter screens at intake, (b) duplication of all pipelines from intake to final distribution lines, (c) provisions for daily flushing of all pipes, (d) continual flushing of sediments from settling tank, (e) final distribution lines and petcocks leave from the upper sides of secondary supply lines, and (f) use of straight runs of pipe with no elbows.

2. Air embolism in experimental animals, by (a) use of a gravity system of sea-water distribution, and (b) avoidance of temperature increases by continual flushing of water from the storage tank and by insulating the tank from direct sunlight.

3. Toxicity to experimental animals, corrosion, and erosion, by (a) use of nontoxic plastic pipe and valves, and (b) use of rubber-coated pumps.

We believe that this facility, when completed, will be an important addition to the nation's research facilities. The intensive planning which has resulted in the final flexible design, both of the laboratory and of the sea-water system, should produce a marine, controlled-environment laboratory that will be as useful and convient for scientists of the future as for those of us who will be fortunate enough to use it today.

ADDENDUM

Since this was written, the new installation has been made with duplicate intake and distribution systems. Valves are so arranged that one of the alternate systems is allowed to remain at rest, filled with salt water for a period of 1 or 2 weeks, more than sufficient to kill attached organisms, through anoxia. At the end of this period, the stagnant water, with the remains of dead organisms, is flushed out with clean sea water, and the system is returned to service. Lack of sufficient experience prohibits any statement on successful performance. In the unlikely circumstance that this procedure does not reduce attached growth, provision is made for the introduction of either fresh water or chlorinated water into the resting system.

SALT-WATER SYSTEM AT THE U.S. BIOLOGICAL LABORATORY, BEAUFORT, NORTH CAROLINA

By G. B. Talbot, *Director*

Biological Laboratory, Bureau of Commercial Fisheries, Fish and Wildlife Service,
U.S. Department of the Interior, Beaufort, N.C.

Abstract.—In conjunction with the construction of a modern laboratory at Beaufort, N.C., a new salt-water system was built, since the one in existence, an aged pump-and-tank intermittent facility, had proved inadequate. The system chosen was a continuous-flow type, installed in duplicate, utilizing a 3-horsepower centrifugal pump, flexible polyethylene pipes, and hard-rubber valves. Indoor water tables are constructed of cypress planks lined with sheet lead and of ¾-inch plywood covered with fiberglass. Outdoor tanks are of concrete and may be divided into four compartments by screen partitions. The system has been in continual use since 1955 and provides an uninterrupted flow of salt water, at constant pressure, regardless of valve actuation.

In 1953, with the prospect that a new laboratory was to be constructed at this station to replace the old structure built in 1901, it became necessary to design a new salt-water system for proposed laboratory experiments. The system then in use consisted of a wood tank located on the upper floor of a 2½-story building, and a cast-iron pump with iron pipes and brass valves which supplied salt water intermittently as needed. Since pumping occurred at irregular intervals regardless of the tide, the salinity was apt to change abruptly at each pumping cycle. While this salt-water supply was useful for some purposes, the method of filling was far from satisfactory and the system often drained dry. In addition, the materials used in the construction of this facility were toxic to some marine organisms.

An auxiliary system, consisting of a pump and pressure tank similar to a shallow-well domestic water pump was used, but it had the same disadvantages as the main system, and in addition most marine organisms in the incoming water were killed in the pressure tank. This necessitated cleaning the tank regularly because of the accumulated debris and silt.

Most of the marine stations along the east coast of the United States were visited in an attempt to determine the faults and virtues of existing salt-water systems. In general, the two kinds in use were intermittent and continuous-pumping types. The former has an advantage in that the salinity can be held more or less constant by using an interval timer to regulate the pumping to a definite part of each tidal cycle. In this system a storage tank of sufficient capacity to furnish salt water for all needs for about 12 hours, less pumping time, is necessary. With the continuous-flow system no storage tank is necessary, but tide-linked variations in salinity of the water source cannot be offset by controlled pumping. The choice of systems depends partially upon location

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The author is now Director, Tiburon Marine Laboratory, Bureau of Sport Fisheries and Wildlife, Fish and Wildlife Service, U.S. Department of the Interior, Tiburon, Calif.

of the laboratory in relation to the source of salt water and upon the type of experiments to be conducted.

One major fault found in some continuous-flow systems was that no provision was made to maintain constant pressure when different amounts of water were used. As a result, if small quantities of water were being used in the laboratory and a large valve was opened at an outside tank, pressure in the system dropped to such an extent that water ceased to flow in the laboratory, and it was necessary to further open the spigots in the laboratory to obtain a flow. Then if a valve at an outside tank was closed, pressure in the laboratory increased sufficiently to disrupt experiments in progress. As a result extreme caution had to be observed at all times when valves were opened or closed any place on the system.

Only two really satisfactory nontoxic materials for handling salt-water were found in use in 1953—hard rubber and lead. Some experiments were being carried out with plastic pipe, however, which gave indications that this material would be superior. Since plastic pipe was much more economical to purchase and install than hard rubber and lead, experiments were carried out at this laboratory with several brands of polyethylene and polyvinyl-chloride pipe to determine their toxic effects on marine organisms. Some brands appeared to have an initial toxicity, but after being washed for several hours, none exhibited any toxic properties.

Several pumps were available which had performed satisfactorily on other salt-water systems examined. These were manufactured specifically for handling brines, acids, alkalis, and other corrosive liquids for industrial applications. Construction was of hard rubber or of cast iron lined with rubber so that the liquid being pumped did not come into contact with the metal.

The salt-water system as constructed at this laboratory is a continuous-flow type (shown schematically in figure 1) which was installed in duplicate so that one unit can be used while the other is being cleaned. The dual system also is used to advantage to provide a standby in case of failure in one system. The pump is a 3-horsepower hard-rubber centrifugal type which furnishes 80 gallons per minute at a total head of 30 feet. The same pump is also manufactured in a 1-horsepower model which pumps 40 gallons per

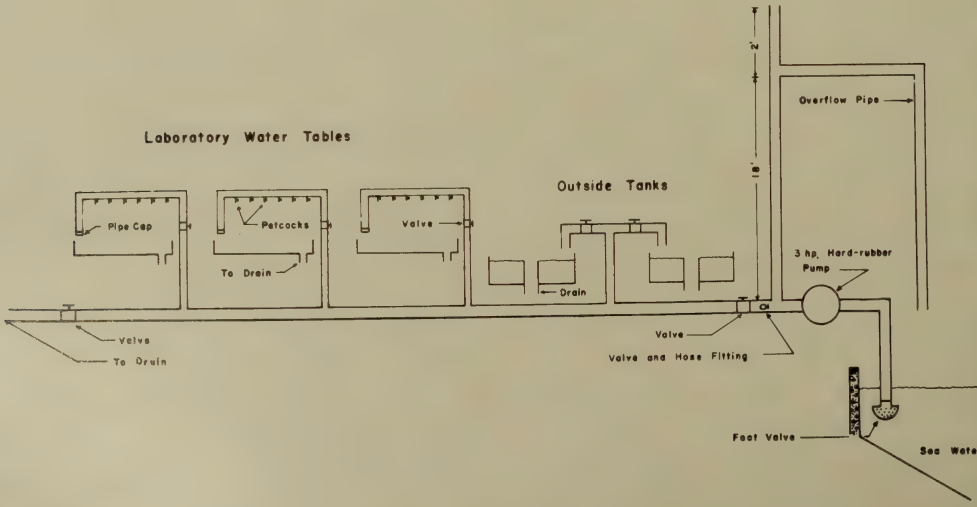

Figure 1.—Salt-water system.

minute at the same head. All pipes are 2-inch flexible polyethylene plastic except at laboratory water tables and outside tanks. Here semirigid plastic pipe is used since it can be tapped for petcocks at the water tables and is self-supporting at the outside tanks. One-inch pipes are used at the laboratory tables, and at the outside tanks 1½-inch pipes are used. The foot valve and pipeline valves are of rigid plastic with polyethylene diaphragms.

An overflow standpipe 18 feet at top elevation above the pump maintains constant pressure in the system regardless of how much water is being used, within the capacity of the pump. Excess water flows from the top of the standpipe, and as long as some water is overflowing, the pressure at all outlets at tanks and water tables remains constant regardless of where valves are opened or closed. The top elevation of the standpipe is 12 feet above the highest outlet on the system. Of course, if outlets at higher elevations are desired, such as in a second-floor laboratory, the height of the standpipe would have to be increased to above the outlet height to increase the pressure so that water would rise to the higher elevation. In our installation the top of the standpipe is extended 2 feet above the overflow pipe to accommodate surges of water when the pump is first started.

Hard-rubber pumps must not be allowed to run dry, as this will ruin the pump shaft and seals. Therefore, as a safety precaution a pressure-operated switch is inserted in the electrical power lines to the pump so that it will be stopped automatically if water is not being pumped. This has proved useful many times when the foot valve has become clogged, as by a piece of plastic sheeting, and also has prevented the pump from restarting when the pump and lines have drained dry after a prolonged power failure.

To facilitate the pumping operation when the lines are empty, a valve with a fitting to which a garden hose can be attached is located near the standpipe. The standpipe, pump, and suction line can then be filled with fresh water from a near-by fresh-water spigot before starting the pump.

Since this system is nontoxic to marine organisms, considerable growth occurs within the plastic pipes, thus reducing the amount of water that is transported. The rate of fouling of salt-water systems probably depends upon latitude of the installation and local conditions. In this area it is necessary to clean the system about twice a year. This is accomplished by stopping the pump and allowing the water to remain in the system for about a week until all organisms are dead. Soft forms usually can then be flushed out by starting the pump, opening all valves and drains, and removing pipe caps from the supply at the water tables.

About once a year the setting of hard-shelled animals, such as oysters and barnacles, clogs the pipe to such an extent that acid must be used to dissolve or loosen them. Commercial hydrochloric acid diluted to about 10 percent (1 part acid, 3 parts water) is introduced into the standpipe, and all outlet valves are opened slightly until the dilute acid reaches all parts of the system. The acid solution is allowed to remain in the pipes for approximately 48 hours and the pipes can then be flushed clean.

The outside tanks are made of concrete with inside measurements of 6 feet by 16 feet with depth of 30 inches at the ends and 31 inches near the center (figs. 2 and 3). Notches on the sides allow vertical screen partitions to be inserted so that the tank can be divided into four compartments. A 4-inch pipe nipple cast into the bottom accommodates standpipe over-

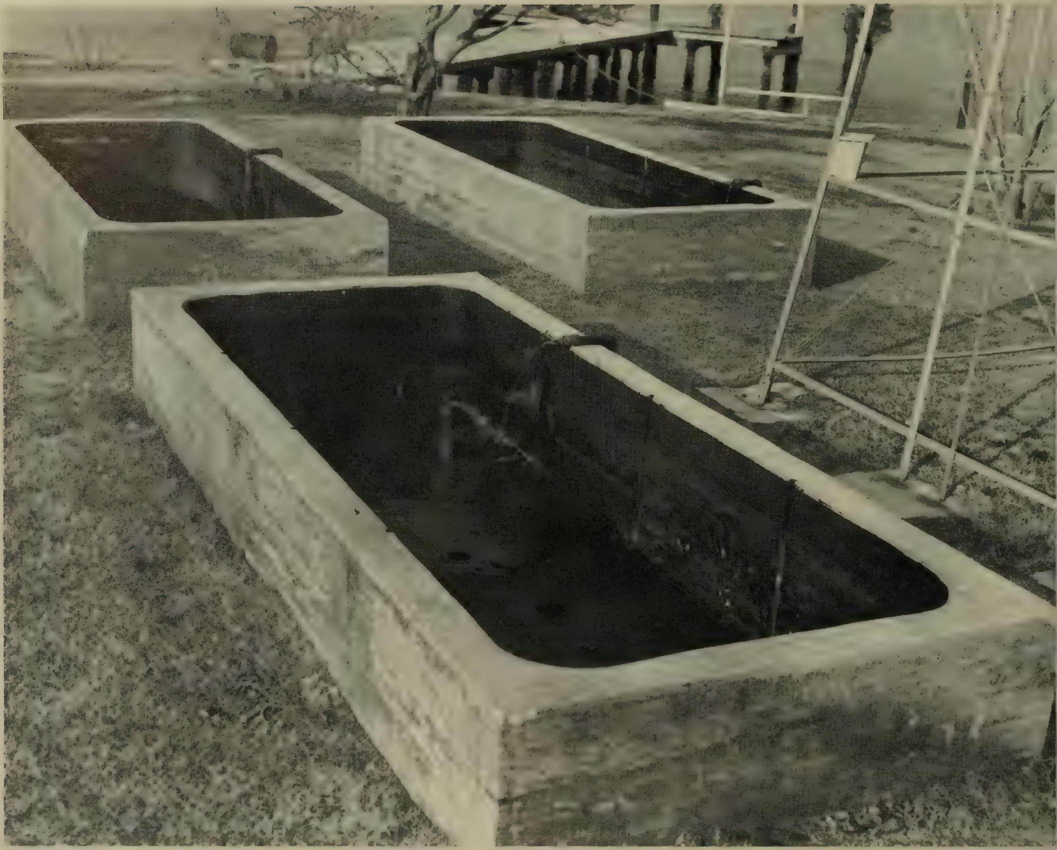

FIGURE 2.—Outside tanks.

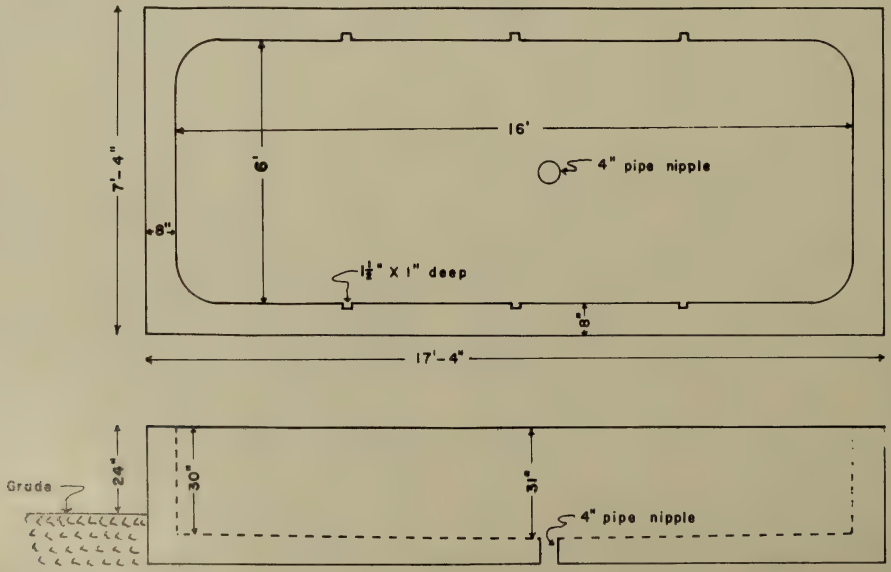

FIGURE 3.—Plan (at top) and cross section (at bottom) of outside tank.

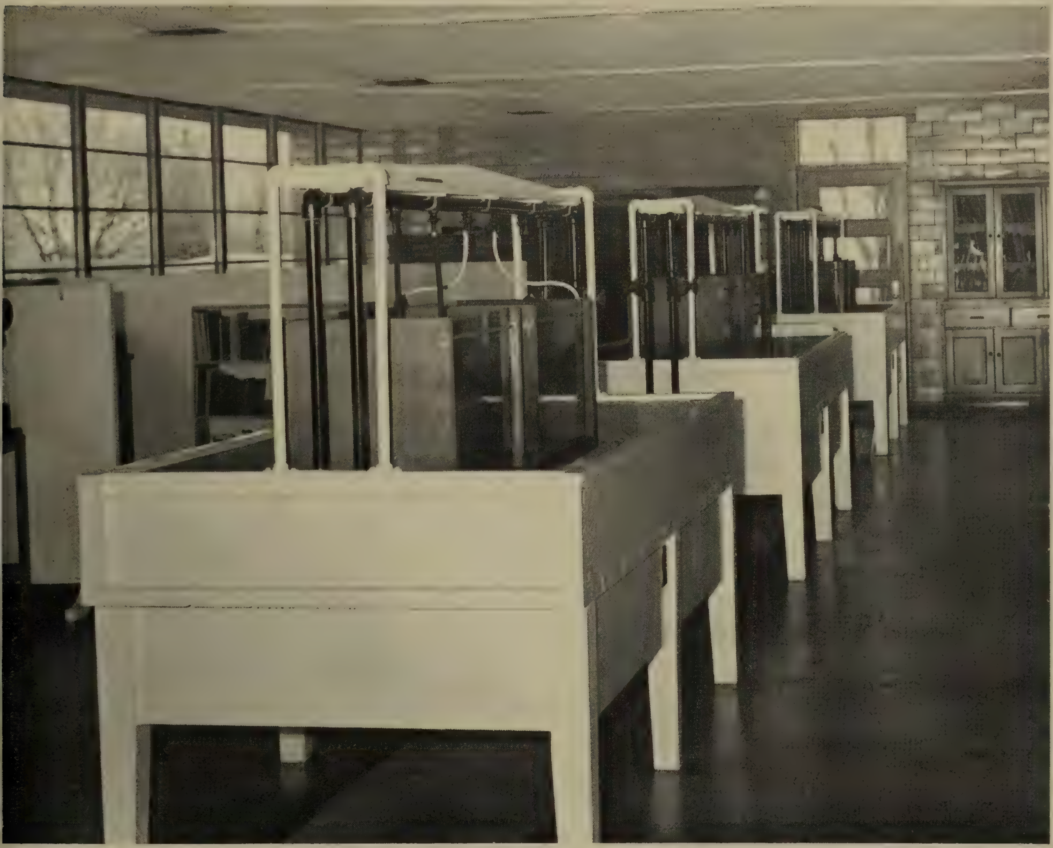

FIGURE 4.—Laboratory water tables with dual salt-water systems.

flows of various heights. The drains from the outside tanks and laboratory tables are of 8-inch terra-cotta pipe with cemented joints.

The water tables in the laboratory, with inside dimensions of 4 feet by 8 feet by 10 inches deep, are constructed of 2- by 10-inch cypress planks and are lined with sheet lead (fig. 4). More recently, additional water tables were constructed of ¾-inch plywood lined with fiberglass. Both types have proved satisfactory. All salt-water pipes as well as other utility lines in the laboratory are located in trenches with sheet iron covers at floor level so that they are readily accessible in case repairs are needed and also to allow flexibility in arrangement of equipment.

This salt-water system has now been in almost continuous operation for more than 7 years and has given excellent service.

SALT-WATER SYSTEM AT THE ST. ANDREWS BIOLOGICAL STATION OF THE FISHERIES RESEARCH BOARD OF CANADA

By D. S. Mann, D. W. McLeese, and L. R. Day

Fisheries Research Board of Canada, Biological Station, St. Andrews, New Brunswick, Canada

Abstract.—Submerged stainless-steel and aluminum-alloy pumps supply salt water through nontoxic plastic piping and fittings to laboratory facilities, and to a 30,000-gallon header tank through a partially effective antisurge unit. Straight runs and T-joints in piping facilitate cleaning. Hot salt water for blending in temperature experiments is produced in a stainless-steel heat exchanger in an oil-fired fresh-water boiler. Expansion valves at compressed-air pumps prevent toxic ions from passing through the copper air-distribution system.

The salt-water system at St. Andrews, New Brunswick, is used to meet various requirements of fishery research, ranging from the culture of planktonic forms to studies of the growth and behaviour of commercial-sized species (fish, crustaceans, mollusks) in controlled environments.

THE SALT-WATER SUPPLY

The station is located where the estuary of the St. Croix River empties into Passamaquoddy Bay and has unlimited access to natural sea water. Each year, the level of diurnal high tides ranges above normal low water from 19 feet during neap tides to 28 feet during spring tides. Water temperatures range from about 32° F. (0° C.) in winter to a maximum of 55° F. (13° C.) in summer. Salinity normally ranges around 28 to 31 parts per thousand although it may fall to 25 parts per thousand during spring runoff. The oxygen content of the sea water is normal.

THE SALT-WATER SYSTEM

The salt-water intake is on the outer face of the station's wharf, approximately 18 feet above the sea bed and 4 feet below the lowest low-tide level. Unfiltered salt water is pumped to a 30,000-gallon concrete reservoir tank about 40 feet above the main laboratories. This elevation provides a constant pressure head for the system. From the reservoir tank, water is distributed to the laboratory buildings (fig. 1). There are facilities to heat part of the supply but no facilities for refrigeration (fig. 2).

Pumps

Two intake pumps, each of 100 gallons per minute capacity, are in use. They are submersible, "Flygt" model B–80L pumps, manufactured by the Stenburg Corporation, Stockholm, Sweden. These pumps are constructed of a salt-water-resistant aluminum alloy with a stainless-steel impeller. Experience has shown that stainless steel is less subject to corrosion and breakage than cast iron. Because the pumps are submerged, leaks at the suction end, which can cause the water to be supersaturated with gases, are avoided. The two pumps have been in use since 1958—one pump in use while the other was being

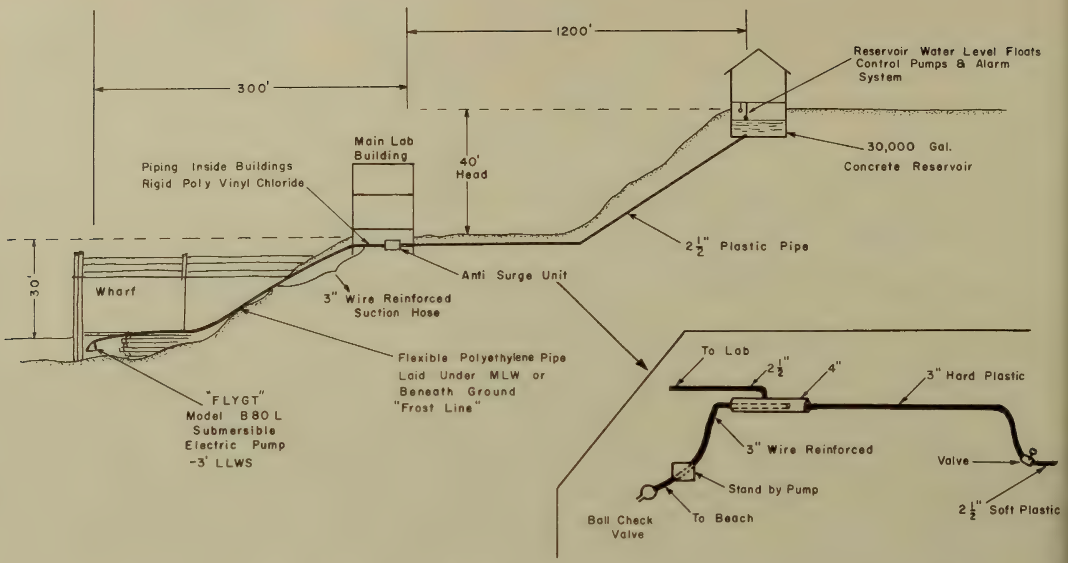
1200'
300'
Reservoir Water Level Floats Control Pumps & Alarm System
30,000 Gal. Concrete Reservoir
Main Lab Building
40' Head
Piping Inside Buildings Rigid Poly Vinyl Chloride
2½" Plastic Pipe
30'
Wharf
Anti Surge Unit
3" Wire Reinforced Suction Hose
Flexible Polyethylene Pipe Laid Under MLW or Beneath Ground "Frost Line"
"FLYGT" Model B 80 L Submersible Electric Pump -3' LLWS
To Lab
2½"
4"
3" Hard Plastic
3" Wire Reinforced
Valve
Stand by Pump
2½" Soft Plastic
Ball Check Valve
To Beach

FIGURE 1.—Salt-water system at St. Andrews, N.B., with insert of antisurge unit.

serviced or held in reserve as a standby. Normally the pumps are interchanged on a 6 months' basis. In case of breakdown, pumps can be replaced in less than 1 hour by two men at the first low tide. A 3-inch line from the pump joins a single 2½-inch flexible polyethylene line leading to the reservoir and main laboratory. To reduce pressure surge when the intake pump operates, an antisurge unit is built into the system at the junction of the three branches of this water line (fig. 1). To eliminate surge, a separate line is being laid from the intake pump to the reservoir.

A turbine-type pump of smaller capacity (80 gallons per minute) was formerly used. This type also provided pro-

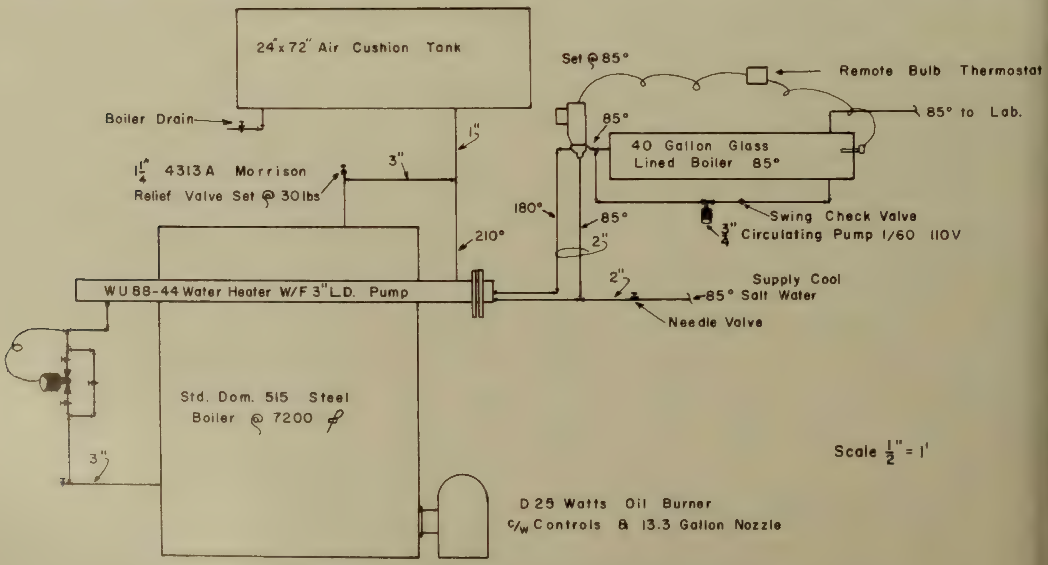
24"x 72" Air Cushion Tank
Boiler Drain
1½"
1¼" 4313 A Morrison Relief Valve Set @ 30 lbs
3"
Set @ 85°
Remote Bulb Thermostat
85° to Lab.
85°
40 Gallon Glass Lined Boiler 85°
180°
85°
2½"
210°
Swing Check Valve
¾" Circulating Pump 1/60 110V
WU 88-44 Water Heater W/F 3" I.D. Pump
2"
Supply Cool 85° Salt Water
Needle Valve
Std. Dom. 515 Steel Boiler @ 7200 #
3"
Scale ½" = 1'
D 25 Watts Oil Burner C/w Controls & 13.3 Gallon Nozzle

FIGURE 2.—Diagram of salt-water furnace.

tection from supersaturation with gases. Although it was generally satisfactory, the rigid intake pipe enclosing the propeller shaft must be protected from damage from such things as boats, drift ice, and other causes. For example, the shaft of the turbine pump was severely damaged when the former wharf settled suddenly. An enclosed pumphouse is also required.

To reduce the possibility of contamination from metal ions, the use of metal is avoided whenever possible (figs. 3 and 4).

Piping

Plastic piping of polyethylene and polyvinyl chloride (PVC) is used throughout the laboratory buildings. There has been no evidence to suggest that either material is toxic. The underground piping is flexible polyethylene and is laid below frost level. Rigid piping is used in the laboratories because fewer supports are required and the sidewalls of the pipes can be tapped to receive stopcocks where required.

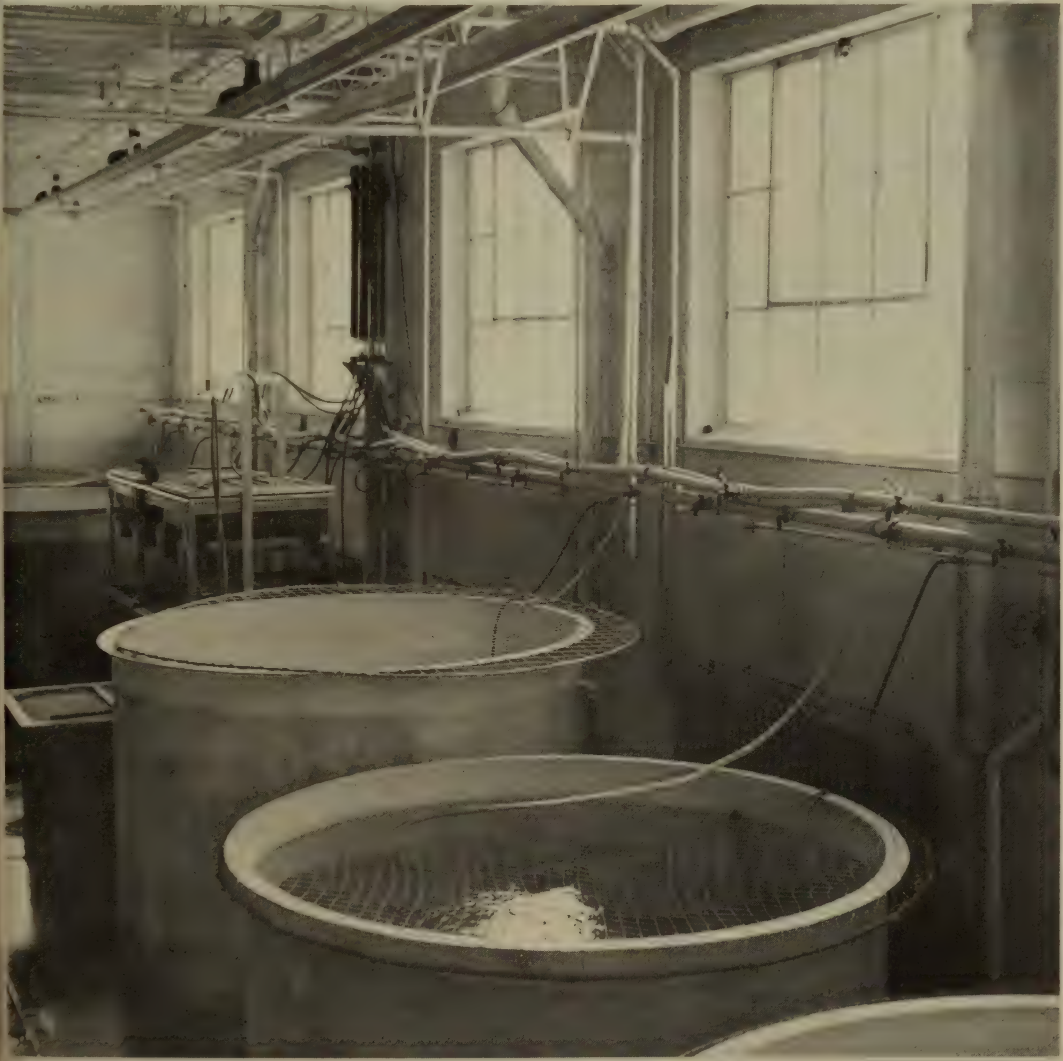

FIGURE 3.—Hot and cold salt-water, fresh-water, and compressed-air piping along the west wall of the basement laboratory.

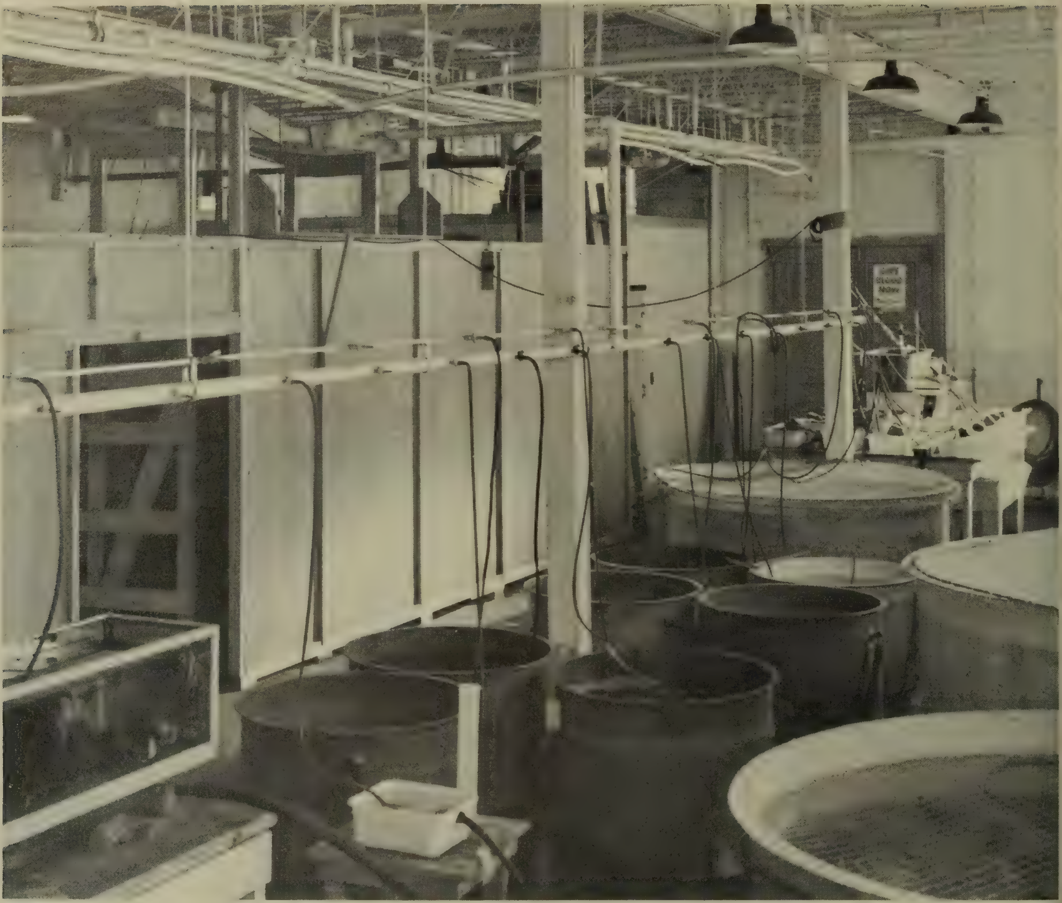

FIGURE 4.—Overhead salt-water and compressed-air lines in the basement laboratory.

Valves and connectors throughout the system are of nontoxic plastic materials, usually rigid PVC but in some cases a blend of styrene acrylonitrile-butadiene copolymer, sold under the trade name Uscolite.

The most satisfactory valve design used to date in our experience is a ball valve of rigid PVC using soft-seal seat rings of Teflon. This valve does not stick even after prolonged periods in either the open or closed position. Only a quarter turn is required to close the valve. No pressure is needed to effect a tight seal.

All piping is black or gray to exclude light and thereby inhibit seaweed growth. Differently colored pipes can be used as a key or code to the contents and supply points. Fouling by mussel growth and occasional clogging with silt and sand are continuing problems. To reduce clogging and to facilitate cleanout, straight runs of pipe are used wherever possible. Cleanouts (tees instead of 90-degree elbows) and break-away couplings are provided at the ends of straight runs of pipe. Although considerable cleanout is accomplished by flushing, it is sometimes necessary to clear sections of the pipes with mechanical reamers. Numerous shutoffs are located at strategic places to allow partial shutdown to repair, to clean out, or to extend the lines. Wherever possible, pipes and fittings are exposed and readily accessible.

Routine late-fall cleanout eliminates much of the yearly accumulation of mussels and silt.

Condensation on pipes conducting cool water throughout the laboratory is troublesome, particularly during hot humid weather. Aside from personal annoyance, drip from overhead pipes on electrical equipment is dangerous. Pipes distributed through laboratory areas should be insulated.

HEATING AND OTHER FACILITIES

The heater system consists of a standard heating boiler of the horizontal return tube type, fired by a thermostatically controlled oil burner. The fresh-water boiler output feeds into the shell of the heat exchange. Heat is transferred to salt water contained within stainless-steel (type 316) heat-exchange tubes. The salt water leaving the exchanger is heated to 180° F. This 180° F. salt water is in turn mixed with cool sea water by means of an automatic motorized 3-way valve to produce salt water at 85° F. for distribution to the laboratories. The system has the capacity to heat 20 gallons per minute of salt water from 32° F. to 85° F. (fig. 2). Required temperatures at the experimental tanks are controlled by blending hot (85° F. water) and cool sea water to achieve the desired temperatures.

Thermoregulators

Thermoregulators, relays, and small pumps for either hot or cool sea water are used on individual tanks to overcome temperature fluctuations resulting from slight changes in flow, changes in room temperature, and changes in temperature of the cool sea water. By this means, it is possible to control temperatures to within 0.2° F. in tanks of 100- to 200-gallon capacity without insulation. This system eliminates the use of dangerous electrical immersion heaters.

Aeration

Two interconnected 5-horsepower De-Vilbiss air compressors supply compressed air for aeration of the water in individual tanks. Copper piping distributes the compressed air around the laboratories. To avoid carryover of any toxic metal ions, an expansion valve at the outlet of the compressor dries the air as it leaves the compressor. Additional drying tubes can be fitted to individual outlets when necessary. Compressed air is distributed in the experimental tanks with "air diffuser" or "air breaker stones."

Refrigeration

Special facilities to refrigerate salt water have not yet been acquired. Low temperatures are obtained naturally during the winter months. Some individual tanks are maintained in a controlled-temperature walk-in cold room. With no flow of water through tanks in the cold room, the water is aerated to maintain suitable oxygen content. A small portable refrigeration unit with a stainless-steel cooling coil is used to cool single experimental tanks.

Reduced salinity

When required, reduced salinities are obtained for individual experimental tanks by mixing suitable constant flows of salt and fresh water.

Filtration

When required, filters with disposable cellulose cartridges are used on the water supply to individual tanks.

SPECIAL PROBLEMS

Electrical-power or equipment failure has been anticipated at St. Andrews. Auxiliary generating equipment has not been installed to date but a gasoline-driven emergency pump and a standby electric pump are available. In addition, the reservoir has sufficient capacity to maintain flows of water to the experimental and storage tanks for several hours. Provided there is no electrical failure, animals can be kept alive during breakdowns in the pumping system by aeration in individual tanks with compressed air. In an emergency, the salt-water fire-fighting unit can be used to maintain the required level of sea water in the reservoir.

Although two possible causes of supersaturation with gases are eliminated in our system by using a submersible pump and avoiding air-cushion pressure tanks, heated water from the furnace equipment may be supersaturated at times. To overcome this, equilibration columns are used for individual tanks or groups of tanks. Similar columns, using nitrogen from cylinders of compressed gas, are used to provide water with a low content of dissolved oxygen for special experiments.

ALARMS

At St. Andrews, an alarm system signals when water levels in the reservoir fall below a predetermined level indicating a pump or waterline failure. A second alarm system monitors water supply to experimental tanks. Alarms at the tanks can be at the discretion of the researcher. At St. Andrews, alarms are incorporated in an automatic, multipoint temperature-recording system.

SUMMARY

At the present stage of evolution of the salt-water system at the St. Andrews Station, certain difficulties have been overcome.

Submersible pumps eliminate air leaks at the suction end, a major cause of supersaturation with gases. Except for the aluminum and stainless steel in the pumps, all metals have been eliminated by use of plastic pipes and fittings.

The reservoir tank provides an almost constant pressure head and, in addition, a reserve supply for several hours in case of breakdown.

Thermostatically controlled heated sea water maintained by an oil-fired furnace eliminates practically all potentially dangerous electrical equipment in and around the experimental tanks.

Although compressed air for aeration is distributed in copper pipes, an expansion valve dries the air as it leaves the compressor to eliminate possible carryover of toxic metal ions.

Design of the system for easy cleanout helps to overcome difficulties arising from clogging with mussels and silt.

The system functions well at present, but is not trouble free. Continued improvement is required.

A separate feed line from the reservoir to the laboratories is planned to overcome present surge difficulties.

Greater duplication of the system is required, including greater attention to placement details for readily accessible shutoffs and convenient shunt lines.

Partial filtration of the system is required to combat occasional heavy silting caused by wave action on the beach or heavy rains. As an alternative, relocation of the intake into deeper offshore water where there may be less silt is being considered.

All the water from the tanks runs to waste. Although possibly desirable, the furnace heater is not equipped with a feedback system to conserve energy.

Refrigeration of part of the supply is desirable to extend the scope or flexibility of the maintained environmental conditions.

Insulation of pipes, fittings, and tanks would be an improvement for easier maintenance of required conditions, for conserving energy, and to increase the safety standards of the establishment.

Standby generating equipment is required to maintain basic facilities during periods of power failure.

SEA-WATER SYSTEMS AT THE FRIDAY HARBOR LABORATORIES OF THE UNIVERSITY OF WASHINGTON

By Robert L. Fernald, *Director*

Friday Harbor Laboratories, University of Washington, San Juan Island, Wash.

Abstract.—The Friday Harbor Laboratories are on San Juan Island, Wash., in an area where the sea water is relatively free from industrial pollution and fresh-water runoff. Three separate sea-water systems are in operation. Two have glass piping over the span of 1,500 feet from pumps to laboratories; the third has polyethylene piping. Each has a hard-rubber centrifugal pump; a 250-gallon, glass-lined receiving tank maintains a constant head of pressure in the laboratories. The water enters the laboratories with little or no change in temperature, or fauna.

The field laboratories for marine sciences at the University of Washington, the Friday Harbor Laboratories, are located on some 484 acres of shore property on San Juan Island, Wash., approximately 80 miles to the north of Seattle. The Laboratories were established in this general area in 1904, and development on the present site began in 1924. In recent years, the past 12, we have had some experience in the design, construction, and operation of sea-water systems which may prove of interest to similar laboratory operations.

At present we have in operation three more-or-less separate and independent sea-water systems. However, two were designed in such fashion that the lines may be interconnected and the water mixed in delivery to the several smaller laboratory buildings. The three systems have a number of features in common. All draw water from a single location marked by the "cantilever pier" shown in figure 1, on a point at the entrance to Friday Harbor proper and projecting into San Juan Channel along the east side of San Juan Island. The sea water at this site is highly stable in temperature, is well mixed, and is free of pollution and fresh-water runoff. The intakes are in the rapidly moving current of sea water just below extreme low water level. It is anticipated that in the near future the intakes will be lowered an additional 5 feet to reduce clogging by free-floating *Ulva* and other algae in late summer. Each intake by way of a 3-inch hard-rubber foot valve is protected by a screen of polyethylene.

In addition the water in each system is pumped by a hard-rubber-lined centrifugal pump powered by an electric motor. Both 5-hp. and 7½-hp. motors are in use and are operated continuously when the system is functioning. The suction lift is approximately 15 feet at low water. In each system the water is carried some 1,500 feet to the laboratory area where it is delivered to a 200-gallon glass-lined tank outside the laboratories. The three receiving tanks are of the same small size to avoid holding the water for any appreciable period of time and thus risk marked change in temperature of the water on unusually cold or hot days. Each receiving

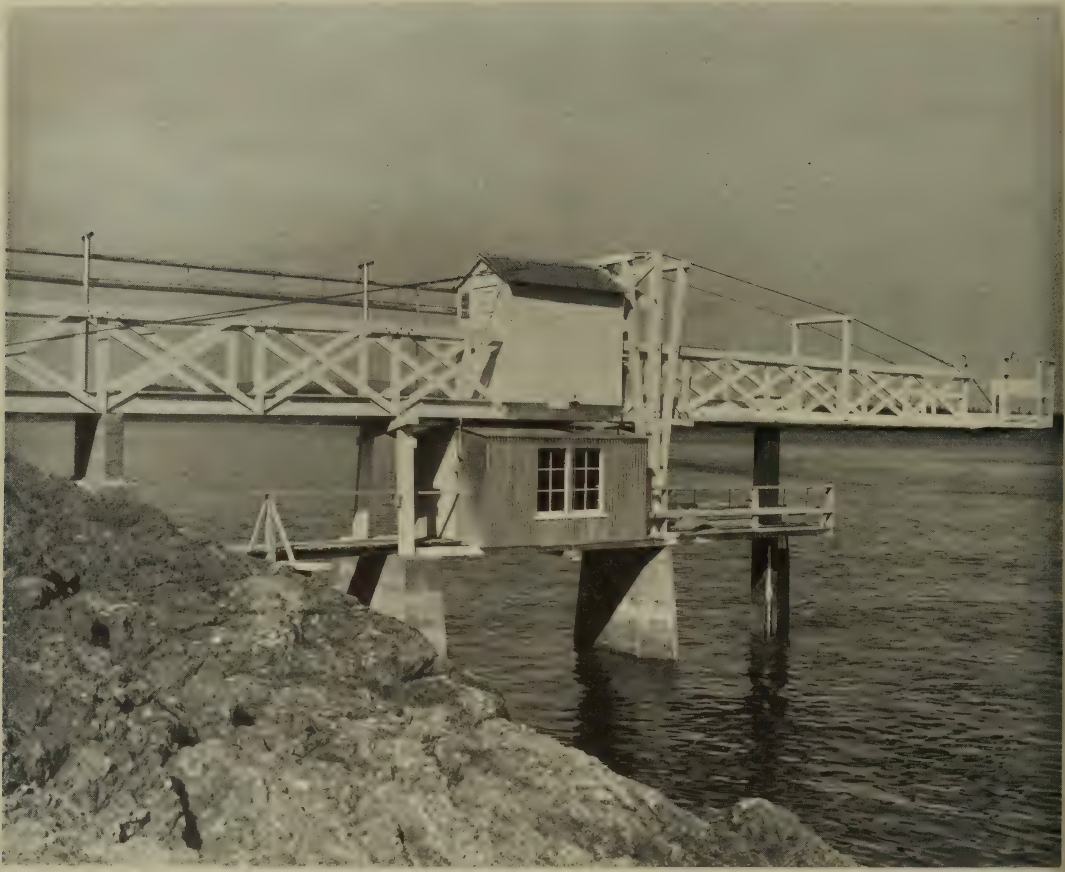

FIGURE 1.—Cantilever pier and pumphouse.

tank is approximately 15 feet above the level of delivery to the sea-water tables and tanks in the laboratories; the tank thus acts as a basis for a controlled gravity-feed system to the tables and aquariums. One of these receiving tanks manufactured by the Pfaudler Company is shown in figure 2.

The lines to the laboratories and the distribution system within the laboratories are of Pyrex glass pipe with standard rubber gaskets and aluminum flanges. The outlets to the individual sea-water tables and aquariums are devised from regular 4- and 6-mm. laboratory stopcocks. The assembly for the stopcocks is illustrated in figure 3. These have proven to be highly satisfactory and far less expensive than other commercially available glass spigots.

The systems differ in the type of piping used to conduct the water from the pumping station to the receiving tanks. The oldest system, installed in 1950, and the newest, installed in 1962, are of Pyrex glass pipe, 2 and 3 inches in diameter respectively. Each of these lines is insulated against temperature variation by enclosure in a sheeting of aluminum over a ½-inch thickness of glass wool in the 2-inch line and foam rubber of similar thickness in the 3-inch line. This insulation insures delivery to the sea-water table of sea water of a temperature essentially characteristic of that in the channel from which it was taken. On the hottest days of the summer, when the temperature is approximately 90° F., the change in temperature of the sea water is less than 0.5°

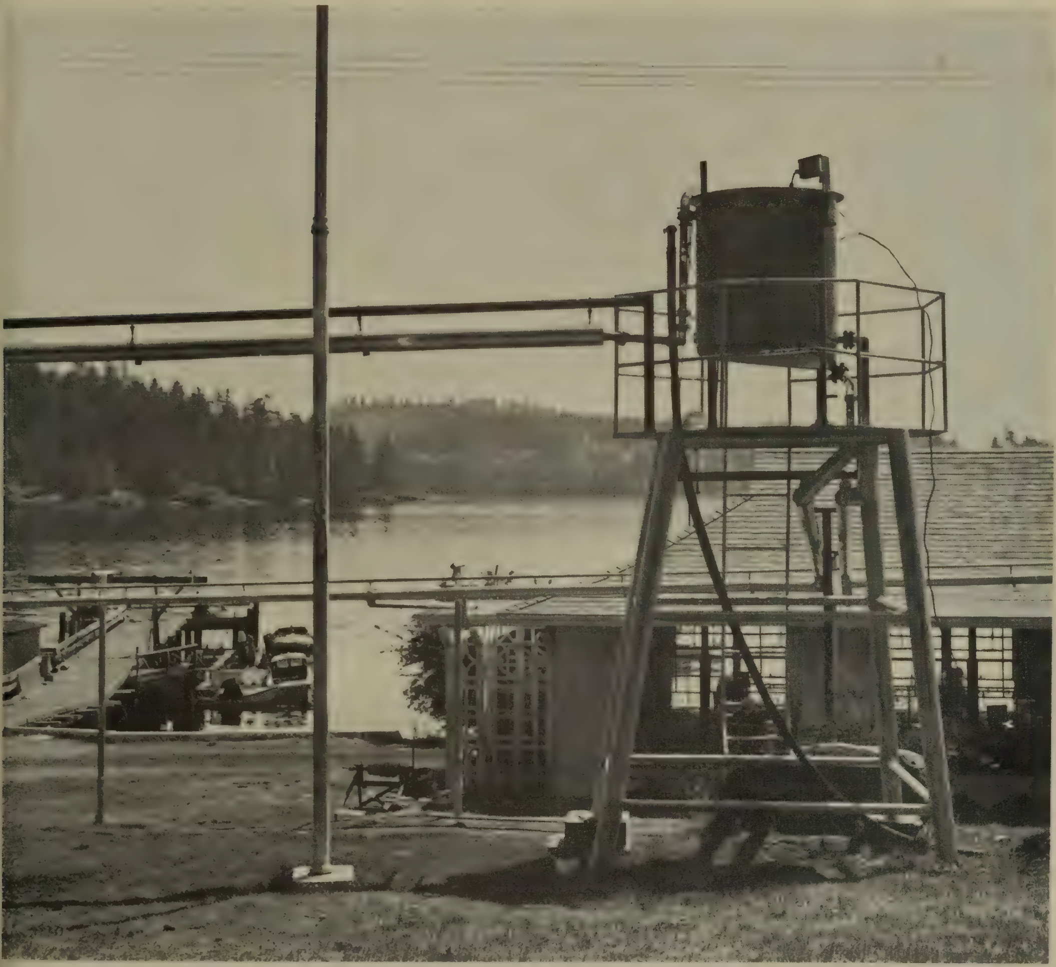

FIGURE 2.—Receiving tank.

C. as it travels to the laboratory. The insulation for the 2-inch line is shown in figure 4. The remaining system was installed in 1959 of 3-inch polyethylene piping. This is virgin grade with a pressure test of 75 pounds per square inch and approved as completely nontoxic by the National Sanitation Foundation. This line was not insulated but has been painted with aluminum paint. Temperature change in the water along this line in hot weather may be as much as 2° C.

Our aquariums and water tables in the laboratories are of two types. In the older systems they have been constructed from 2-inch Douglas-fir, assembled in such fashion that no metal of nails or screws is in contact with the sea water. The wood is untreated and becomes infested with *Bankia* and *Limnoria* each season, but these have been controlled by rotation of use or intermittent use so that only a few of the tables and aquariums have required replacement in the period of almost 12 years. The aquariums are of simple design; in most cases they are approximately 50 by 24 by 12 inches and the sea-water tables are approximately 50 by 42 by 4 inches. They are supported on frames constructed from 1½-inch iron pipe,

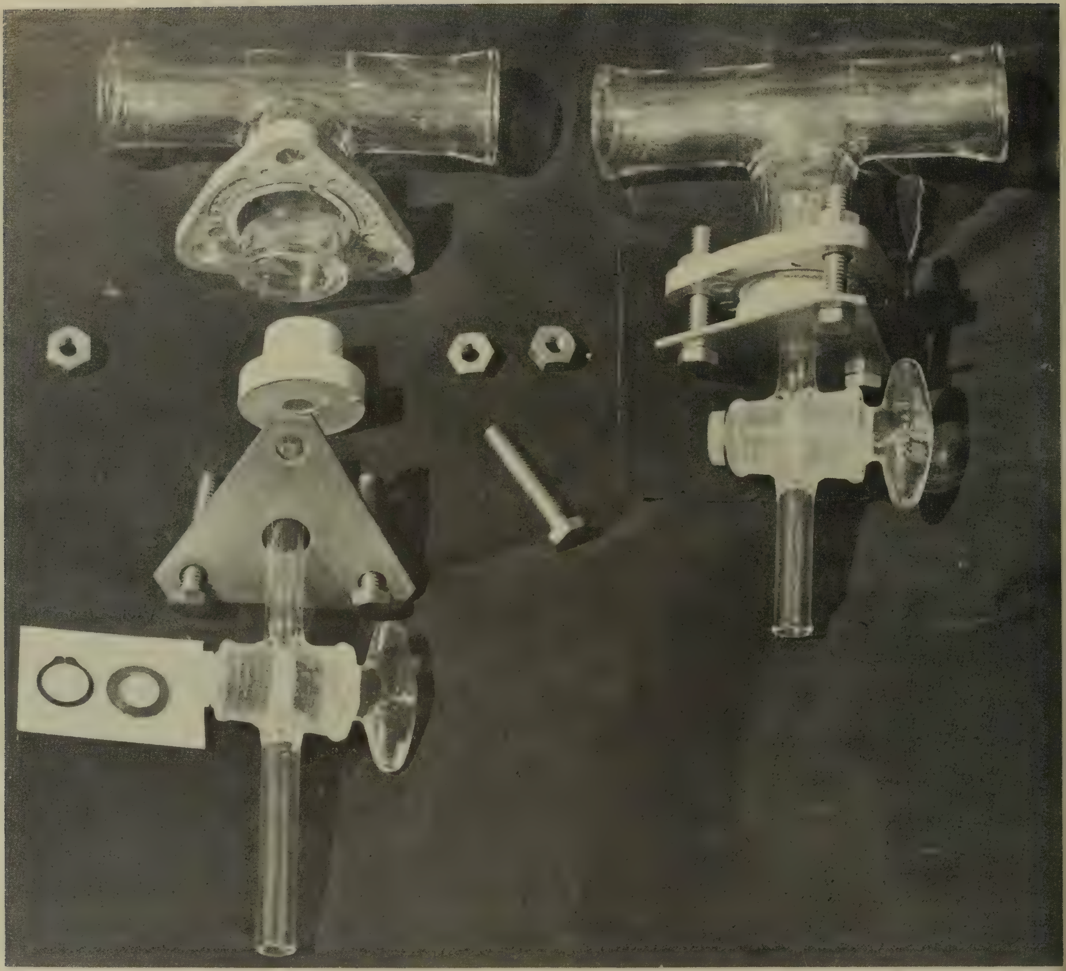

FIGURE 3.—Pyrex stopcock.

welded in the appropriate design and painted with rustproof paint (see fig. 5).

In the research laboratory recently completed, the aquariums and sea-water tables have ⅜-inch plexiglass sides and bottoms. The tanks are supported on the pipe frames on sheets of ¾-inch marine plywood to avoid putting stress on the plexiglass. In addition, the new research building is equipped with a few reinforced-concrete tanks of different sizes and shapes. Among the more useful are two circular tanks 8 feet in diameter and 30 inches deep. These are especially well adapted for the maintenance of more rapidly moving and swimming animals such as squid and fish. Another concrete tank is a large rectangle, 5 by 10 feet, varying in depth from 1 to 3 feet. Other concrete tanks are developed as double-deck water tables, 3 feet wide, 8 inches deep, and as long as 14 feet, with 17 inches' clearance between the two tanks.

In carefully conducted tests to determine the suitability of a wide variety of plastics and substitute piping materials for use in sea-water systems, we found nothing more satisfactory than Pyrex glass. Many materials proved quite unsatisfactory and some essentially useless.

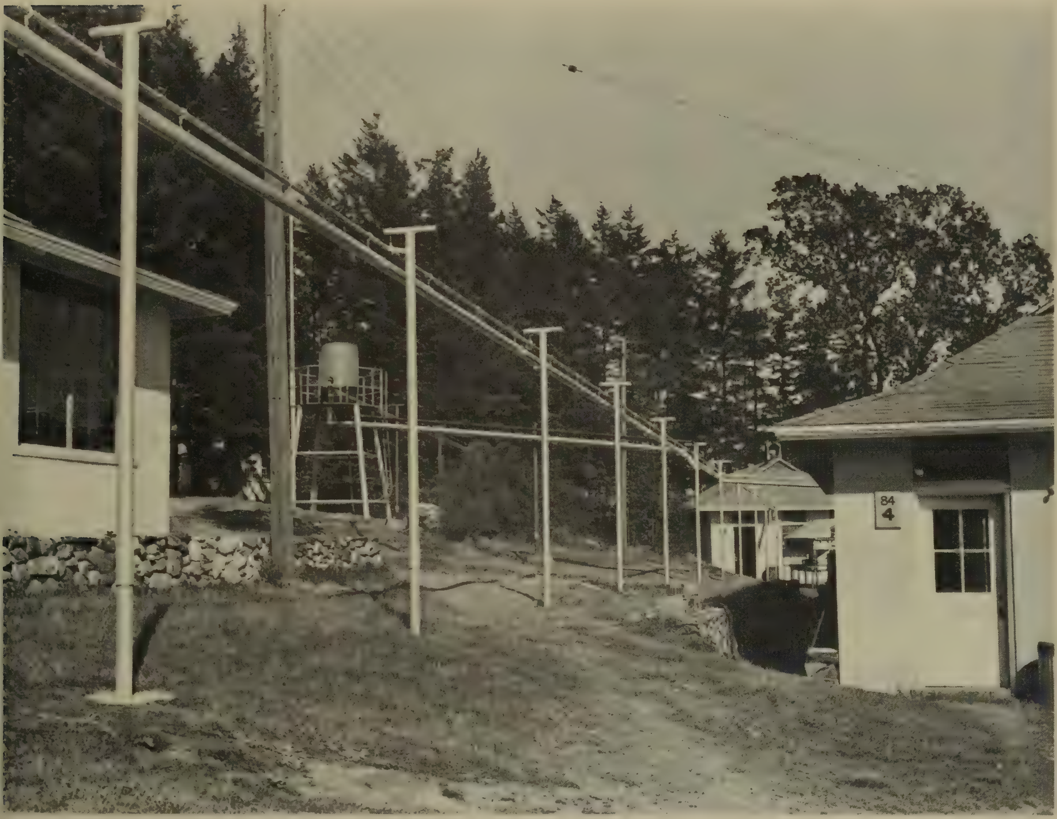

FIGURE 4.—Insulated glass line from cantilever pier to receiving tanks.

Polyethylene of virgin grade and highest quality, while sometimes varying from one production lot to another, proved superior to other commonly recommended plastics. While many of these plastics are advertised as "nontoxic to humans" and appear to have no obviously deleterious effect on many adult marine invertebrates, they may block developmental stages or cause serious abnormalities of differentiation.

Developing eggs and embryos of the purple sea urchin, *Stronglyocentrotus purpuratus*, have been used to real advantage as test subjects to determine the toxicity of various possible materials for use in sea-water systems. Since they have been used extensively as experimental material in this laboratory, their development is very well known and any variation from the normal in rate or kind of development can be readily detected. In addition they are quite sensitive to heavy metals and other toxic agents and the presence of minute quantities of such is usually reflected in dramatic change in the normal pattern of development.

As will have been noted, there is no filtering of the sea water pumped through our systems. The water arrives at the tanks with the usual complement of planktonic organisms, and plankton feeders thrive in the tanks and aquariums. In addition, since the system is essentially free from toxic agents, the walls of the pipes provide settling space for larvae of many sessile forms and other animals taken in at the intake. Thus the system supports a considerable growth of barnacles, mussels, limpets, etc., as operation continues.

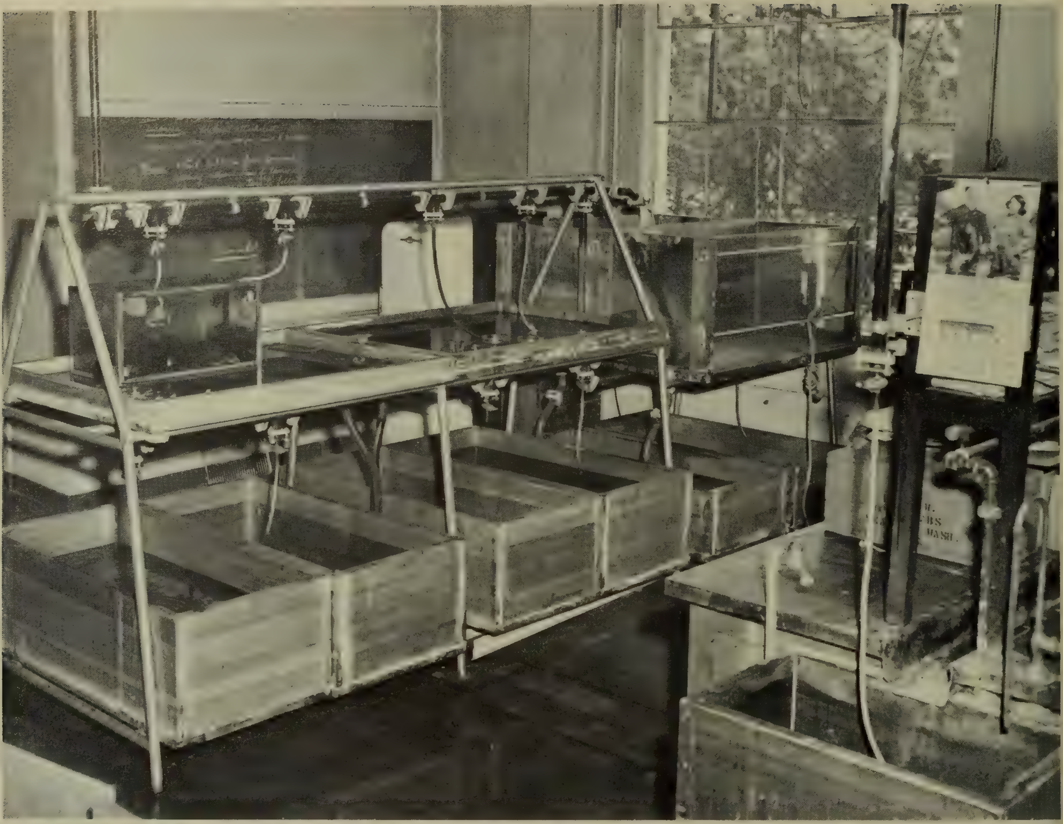

FIGURE 5.—Distribution system, water tables, and aquariums.

Experience has indicated that the best method of removal of excessive growth is by mechanical cleaning of the lines. Fresh water, hot or cold, and acids have proved to be too slow and inefficient to eliminate this growth, and a simple dismantling of certain of the joints followed by pulling of various-sized rubber stoppers through the line has proved more satisfactory and constitutes a less-time-consuming interruption to service. Such a cleaning is required annually throughout the system and more frequently in certain sections. Excessive growth of diatoms within the glass lines has been controlled with some success by covering the lines with sheets of aluminum foil.

In evaluating the systems which we now have in operation at the Friday Harbor Laboratories, there is no question but that the combination of a pump lined with hard rubber, a well-insulated line of Pyrex glass pipe coupled by rubber gaskets, a receiving tank with glass lining, and sea-water tables and aquariums of wood or plexiglass give assurance that the system will deliver water with physical characteristics as nearly like those of the water at the source where the intake operates and as free from contamination with heavy metals as can be desired. In a laboratory such as ours, where the demands for sea water are such that not only must a copious supply be maintained but also it must be nearly the temperature of the channel water and as free from contamination or toxic agents as possible to assure optimal culture conditions for everything from embryos of invertebrates to various algae and fish, the glass systems which we have are excellent.

OREGON FISH COMMISSION'S SALT-WATER SYSTEM AT NEWPORT, OREGON

By C. Dale Snow

Research Laboratory, Oregon Fish Commission, Newport, Oreg.

Abstract.—This paper describes a simple salt-water system used at the Oregon Fish Commission's shellfish-research laboratory at Newport, Oreg., and discusses some of the problems in maintaining and operating such systems.

Our salt-water system at Newport, Oreg., is simple and is illustrated in figure 1. Water is pumped into a 750-gallon Douglas-fir tank that has been treated with biturine to slow down damage by *Teredos* and *Limnoria*. The pump in use is a 1-inch Jabsco powered by a ½-horsepower electric motor, but a Gould pump with glass liner and impeller would be far superior. Pumping is regulated by an Allen-Bradley float switch that can be operated automatically or manually. Placement of the outlet from the tank 6 inches above the bottom reduces the amount of sediment pumped into the aquarium trays. For piping water from the tank to the aquarium we use 1-inch Portco plastic pipe. Aquarium trays are made of ½-inch marine plywood that is also treated with biturine. The water supply into the aquarium enters through one to four tee-joints placed in the salt-water line. Each tee has a rubber stopper with a ¼-inch glass tube through the center and a piece of surgical tubing on the end to which a screw clamp is attached. The amount of water entering is regulated by this clamp and the free fall into the aquarium aids in aeration. A 1-inch hole with a rubber stopper and a ½-inch glass-tube standpipe regulates depth of water. To clean the trays, the stopper is removed and the trays are scrubbed with a heavy brush as the water drains out. This system, while quite simple, has proven to be adequate for our purpose of holding animals (clams, crabs, etc.) for various studies.

The biggest problem with this system has been fouling of the water lines by mussels and barnacles. To combat this we periodically flush the lines with hot water during the spring and summer months to control settling and survival of these animals. If the lines are neglected and growth becomes too heavy then the lines must be disassembled and a half-strength Clorox solution run through the pipe. This solution dissolves the byssal threads of the mussels and allows the mussels to be flushed out. If Clorox is used, the lines must then be thoroughly flushed before they are used again. I would recommend that salt-water lines be put together in such a manner and position that they can be taken apart easily for periodic cleaning.

In our salt-water system the outlet placed well above the bottom has proven adequate in allowing sediments to drop out and it holds to a minimum the sediments entering the aquarium. Periodically we allow the level to drop down so that the bottom sediments can be removed with a garden hose siphon. A recent innovation

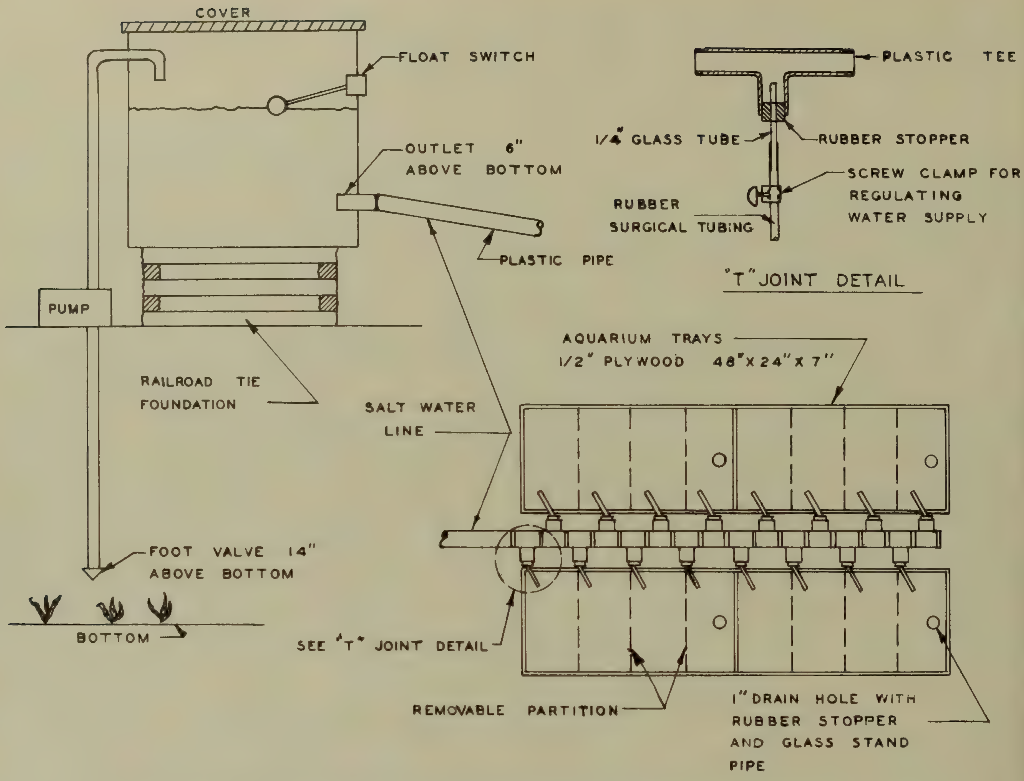

FIGURE 1.—Marine aquarium of the Oregon Fish Commission, Newport, Oreg.

in some water tanks is the lining of the tank with a polyethylene sheet. This not only discourages *Teredos* and *Limnoria* but facilitates cleaning of the tank. Cleaning merely involves removal of the polyethylene which can be hosed off. Unfortunately, with the polyethylene liner you must depend upon siphoning action for your water supply.

Equipment failure has been a minor problem in our system. This is particularly true where the storage tank's capacity is such that it will supply water for several hours. We try to keep a spare motor and impeller for the pump on hand at all times, as these are the two items most likely to cause trouble.

The selection of a pump for a salt-water system depends much on the use to which the water will be put. In our work the 1-inch Jabsco pump has proven highly satisfactory, but for some work this would be inadequate, particularly in oyster or larvae work. The brass liner in this pump can cause trouble. For larval rearing or oyster studies a pump with a glass liner and impeller is far better.

Toxicity of components within the salt-water system is highly important when you are working with larval forms or condition studies. This toxicity can come from a number of sources: (1) The liner or impeller of the pump; (2) materials used to treat the tanks and aquarium trays; and (3) some plastics used for pipes or tank liners. In general it is best to avoid all metals within the system. Plastics such as Portco piping, polyethylene, and some of the new marine resins are nontoxic to most invertebrate larvae and have proven satisfactory in most instances. Cements with high lime contents should be avoided unless the material has been cured for several years in salt water.

I prefer storage tanks as a source of water supply. A tank has several advantages over a continuous-flow pump: (1) better salinity control, (2) water reserve in case of pump failure, and (3) sediment control. In an estuarine situation a continuous-flow pump picks up water at all tide and salinity phases. With a tank you can pump during the high water when salinity is greatest, thus holding salinity at a level you desire. However, wood tanks, if not lined with polyethylene or treated, will be destroyed by marine boring organisms such as *Teredos* or *Limnoria.*

AN ANNULAR TANK FOR SEA FISHES

By Allan C. DeLacy, ***Professor of Fisheries***
University of Washington, Seattle, Wash.

Abstract.—The College of Fisheries of the University of Washington maintains an 8,000-gallon recirculating sea-water system. A 2,000-gallon annular tank has enhanced growth and longevity of certain fish species. Light and temperature control afford flexibility in establishing artificial environments.

The College of Fisheries at the University of Washington maintains a recirculating salt-water system with a total capacity of about 8,000 gallons. Rubber-lined steel pipes, sumps, and storage tanks are prominent features of the system. Twelve rectangular aquariums of concrete and glass, a 2,000-gallon annular tank of rubber-lined steel, and a single 450-gallon fiberglass-lined wooden tank comprise the permanent fish-holding facilities. Additional important features of the system are the sand filters, temperature-control equipment, and flexibility which permits the entire system to be operated as a unit or allows subdivision into two independent systems, one for cooling and one for warming sea-water as desired. Temperatures commonly fluctuate less than 1° F. from a chosen level.

The annular tank (fig. 1) has proved to be a feature of particular interest in the College of Fisheries salt-water system. It is simply a 3-foot-wide annular channel with an outer diameter of 10 feet. A water depth of 4 feet is maintained. Two inlets discharge into the surface at a slight angle thereby producing a weak circular current in the tank. Four evenly spaced surface openings on the inner wall serve as outlets. A bottom outlet is used when the water level is to be lowered. Eight 16-inch-square glass ports in the outer wall and four in the inner wall permit lateral viewing and photography of contained specimens.

Active fish cruise around and around in the annular tank making infrequent contact with the sides. A fingerling silver salmon (*Oncorhynchus kisutch*) was reared to maturity (5 pounds) in the tank without experiencing the deformities and damage to the snout which accompany confinement in the smaller (200-gallon) rectangular aquariums. Two ratfish (*Hydrolagus colliei*) lived 3 years in the tank and finally died after an operational error which permitted a temporary but severe temperature increase. A skilfish (*Erilepis zonifer*) introduced to the tank as a 3-pound, 17-inch fish weighed 36 pounds and measured 39 inches in total length after 6 years.

Total water use by all aquariums averages about 20 gallons a minute. Over a period of several months, salinity may fluctuate through a range of 1 part per thousand, and pH may decrease by a half a unit. Frequency of water replacement is related to type and number of organisms being held but in general from one-half to three-quarters of the water in the system is replaced three or four times a year. New water is brought to the campus by wooden barge from nearby Puget Sound.

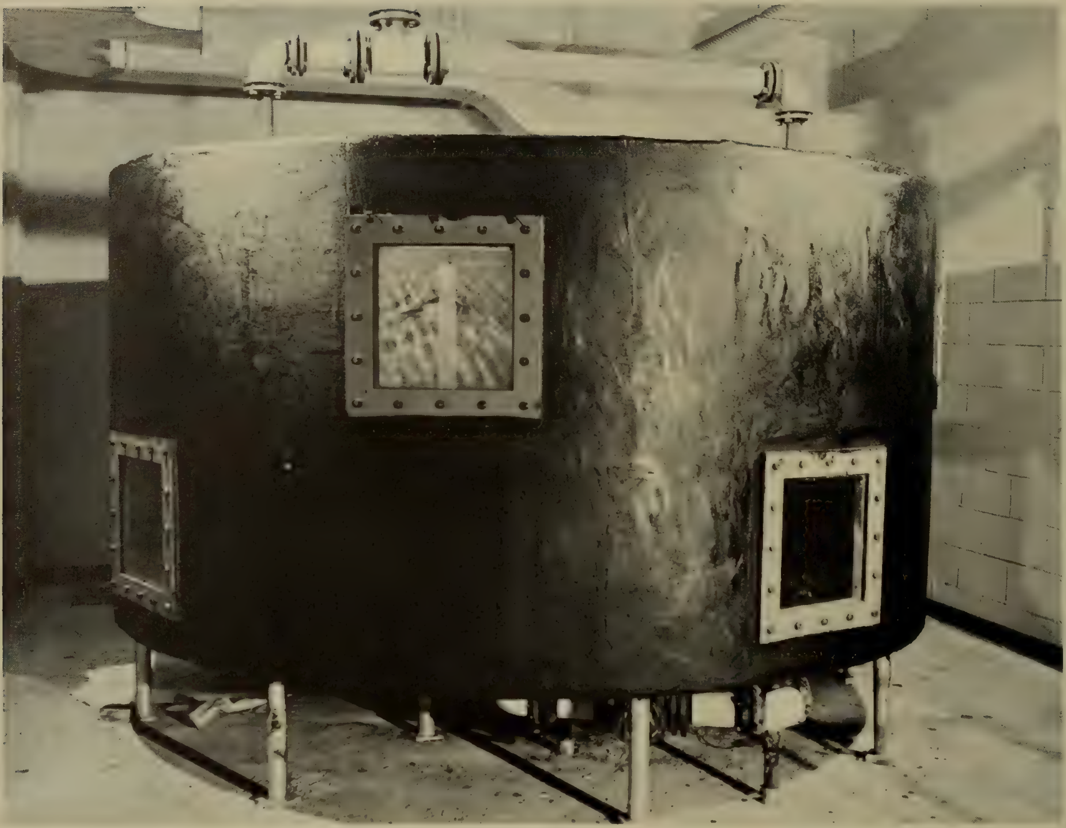

FIGURE 1.—Annular tank at the aquarium of the College of Fisheries, University of Washington, Seattle, Wash.

The sand filters have given satisfactory service since the initial operation of the aquarium system. A gradual decrease in filtration rate usually occurs over a period of several weeks. Occasional stirring of the sand accelerates filtration, but full efficiency is regained only by backwashing the filters. Fresh water is used for backwashing.

An addition to the salt-water system as originally constituted is a fiberglass-lined wooden tank 39 inches wide by 24 inches deep by 10 feet long. The fiberglass lining of the tank proved to be satisfactory and remains in good condition after 42 months of continuous service.

A problem involving fish behavior arose in connection with lighting. All aquariums are in an interior room with no exposure to daylight. Sudden illumination of the room caused some species to dart wildly about, striking sides or bottom of aquariums or sometimes leaping out of the tanks. An effective solution was developed by installation of an auxiliary room light controlled by a time clock which activates a motor-driven rheostat. The light comes on or goes off over a half-hour period, and the duration of "night" and "day" can be adjusted as desired. In practice the time clock is reset several times a year to simulate the lengthening and shortening of days by season.

SEA-WATER SYSTEM OF THE MARINE LABORATORY OF THE UNIVERSITY OF CALIFORNIA, SANTA BARBARA

By Donald K. Joice, Office of Architects and Engineers,
and Demorest Davenport, Department of Biological Sciences,
University of California, Santa Barbara, Calif.

Abstract.—The sea-water intake system of the Marine Laboratory of the University of California, Santa Barbara, lies in the tidal zone of the campus beach. The 48-square-foot filter, a concrete box filled with graded aggregates and having a perforated collection header at the bottom, is located at low-tide level. The filtered water is carried to the laboratory through a buried pipe under the suction of a shore-based jet pump. As a cleaning device, the surf's constant agitation of the beach sand over the filter has contributed to giving an uninterrupted, maintenance-free service of 35 gallons per minute for over 4 years.

The sea-water system of the Marine Laboratory on the campus of the University of California, Santa Barbara, is made up of three basic components: a sand filter intake unit located in the surf approximately 400 feet from the shoreline, a jet-type pump assembly, and a reservoir head tank combination located on shore near the laboratory.

The filter unit has been in operation almost continuously for 4 years without any malfunction, or any maintenance. The pumping portion of the system has required only the type of maintenance and replacement program normal to any pumping plant.

This paper will deal primarily with the sea-water intake unit, which is the critical component of the system. Figure 1 shows the relations of the three components.

The physical characteristics of the campus beach placed certain limitations on the installation. The beach floor is of an impermeable but relatively soft monolithic shale which is known to be over a thousand feet deep. This shale, with a surface elevation of 1.5 feet at the shoreline, tapers off slightly over a foot in 400 feet. From this point it slopes off at a more acute angle, dropping approximately 3 feet in the next 100 feet. In certain winter months the wind and tides clear virtually all sand from the shale leaving it as barren as a table. At such periods the surf comes within a few feet of the shoreline. During the summer months tidal action redeposits the sand on the beach to a depth of from 2 to 6 feet. During these periods the surf will be held out as much as 400 feet from the shoreline.

The intake filter unit is a flat reinforced-concrete box (fig. 1), with 6-inch walls and bottom, 6 feet by 8 feet by 2½ feet inside dimensions. A header made up of 4-inch cast-iron bell-and-spigot crosses runs the length of the box at the bottom. Ten cast-iron perforated laterals, 2 inches in diameter, are lead-caulked into the header. The box is filled with graded aggregate to form the filter, the purpose of which is to screen out sand and flotsam.

The filter unit is buried in the floor of the beach, the top being approximately level with the shale floor at elevation −1.5. The

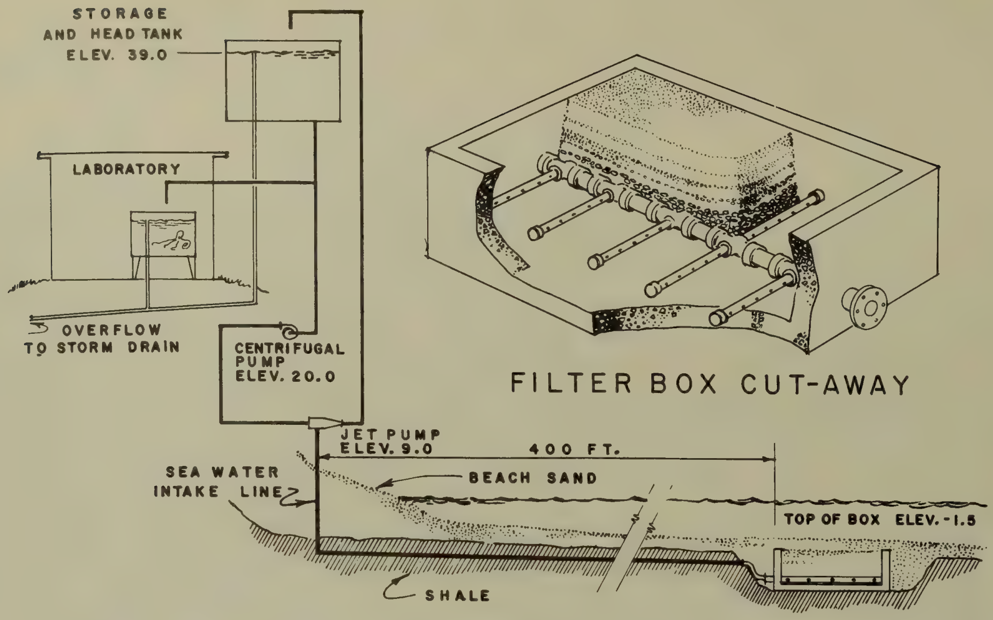

Figure 1.—Piping diagram and cut-away view of filter box in sea-water system of the Marine Laboratory of the University of California, Santa Barbara, Calif.

beach sand covering, ranging in depth from 18 inches to approximately 48 inches, depending on the season, augments the integral filter media of the unit. The area over the filter is covered with water for all but a few of the lowest tides of the year. The unit is connected to the shore installations by a 4-inch asbestos-cement pipe suction line which is also buried and concreted in the beach floor shale, to protect it against tidal action.

The size of the filter unit was determined by a number of factors, all of which were strongly influenced by economic considerations. The prefabricated unit, with 48 square feet of filter area, was designed to provide the 30 gallons per minute required for a small marine laboratory. Weighing approximately 10 tons, it was of a size and shape that could be readily hoisted on and off a truck or trailer.

The total beach installation was completed in 1 day between a high and low tide. As the high tide began to recede, bulldozers followed it out, clearing the 3 feet of sand from the strip which was to be trenched for the pipe installation. As the bulldozers left the shore they were followed by the survey crew, who set grade stakes and were in turn followed by the trenching machine. The pipe-laying crew, assisted by portable pumps to remove seepage from the trench, came only a few yards behind. As soon as four or five lengths of pipe had been installed and fixed in place, transit-mix cement trucks poured concrete over the line. In preparation the bulldozers pushed the sand into revetments along the course of the project. These revetments, which averaged about 8 feet high, enclosed the total work site on the ocean side and served as a cofferdam to hold back the incoming tide late in the afternoon. This gave the construction crew an additional 4 hours of time which the tides would not have otherwise permitted.

A depression approximately 36 inches deep was bulldozed out for the filter location, and the filter was lowered into place. It was then fitted with a reinforced-rubber suction hose, the flexibility of which allowed the final connection to the rigid asbestos-cement pipe line. Bolted flanges were used on these field connections to further speed the installation.

Whereas this filtering system borrowed some of the characteristics of the rapid sand filter used for industrial and municipal water handling, the controlled conditions under which a rapid sand filter is backwashed and maintained were considered impossible to attain in a varying tidal zone situation. It was therefore determined to establish a rate of flow which would be so slow that the dirt absorption in the filter would be negligible. By utilizing this reduced flow it was assumed that air bubbles which result from the negative pressures developed in high-rate filters would not collect to halt the flow. In a small test filter model 1 foot thick it was observed that the rate of water flow through local beach sand was slightly over 1 gallon per minute per square foot under a head of 1 foot. Knowing that there would be tidal heads of up to 8 feet over the filter, which would increase its efficiency, a design rate of 0.65 gallon of water per square foot per minute appeared safe. This rate has been maintained for 4 years of operation with no evidence of a decrease.

The only apparent explanation for the lack of servicing demanded by this system is its location in the surf. It is situated at a point where there is nearly always a strong tidal action and constant movement of sand over it. This sand movement, it is believed, has served as an automatic filter-cleaning device. The first 18 inches of beach sand immediately covering the unit is thought to remain relatively undisturbed except for heavy storms of a severity which is expected only once in several years. The sand above this point is known to be constantly shifting under the normal movement of the surf.

Although it was assumed during the system's design that the shifting of the beach sand would help maintain the filtering qualities of the unit, it was also presumed that the periodic replacement of the surface material would be relatively inexpensive if trouble developed. A south-coast oil refinery sea-water intake system which utilized a beach-sand filter operated successfully for about 2 years before becoming sealed up by accumulation of organisms in the sand above it. This unit, which was nonportable, was located near the shoreline where there was only an occasional tidal flow over it. It depended largely on lateral infiltration through the sand. It was reported to have been maintained in operation after the first stoppage by a periodic chlorine sprinkling treatment of the sand over the filter. In anticipation of such a development the UCSB installation was provided with a connection to the campus domestic water system. It was assumed that a fresh-water back-rinse would help control such growths. None have been detected to date, and the sea water in the laboratory is to all intents and purposes plankton free.

The choice of a pumping system as in the case of the filters was determined in part by the limited finances available. The most economical installation on a first-cost basis was considered to be a jet-pump installation. This system consists of a high-head centrifugal pump energizing a venturi which creates the negative head to draw up the ocean water. The centrifugal pump operates in a loop arrangement. It draws its water from the head tank, discharges it through the venturi back into the head tank. For each gallon of water pumped through the venturi approximately 1 gallon of fresh sea water is drawn in from the ocean and discharged into the head tank. The head tank holds 2,000 gallons of sea water which will normally supply the laboratory and the recirculating centrifugal pump for several hours even though the sea-water intake is temporarily interrupted by a low tide. As the rising tide covers the filter unit the suction from the venturi automatically reestablishes the sea-water flow. The centrifugal pump is protected from running dry by a float switch in the head tank which breaks the circuit in the event the tank water level drops too low.

The economy of the jet system stems from its versatility of location. It can be located considerably above the source water level and still afford a reliable automatic priming function. To have installed a centrifugal turbine pump, which, budget permitting, would have been seriously considered, the pump would have had to be located at elevation 1.5, the terminal level of the pipe from the filter unit. Priming could have been assured only by having the pump at this elevation, which is below the level of the ocean. The higher comparative cost of this installation would have developed from the pump house itself. Instead of being located in a frame structure on the shore at elevation 9.0 as was the jet-pump installation, the turbine pump would have required a reinforced-concrete vault with foundations at elevation −4.5.

Although the simplicity of the jet-pump automatic priming and control system presents great advantages, in view of the demands made upon it by the specific environmental conditions, the relative inefficiency of its operation as compared to a turbine pump should be considered. For example, in the system exhibited in figure 1, a 10-horsepower motor for the jet-pump system is required to deliver 30 gallons per minute of ocean water, whereas a 1-horsepower motor would accomplish the same work with a direct turbine pumping unit. This ratio of 10 to 1, if reflected in electrical energy costs, becomes highly significant in equipment that operates continuously.

The above described system at present serves a small temporary laboratory containing approximately twenty 10-gallon and eight 25-gallon aquariums. There are three large table aquariums receiving running sea water in an outdoor installation.

Building schedules call for a tenfold expansion of present facilities in a permanent building, partially funded by the National Science Foundation, by June of 1964. All laboratories will be provided with running sea water. The sea-water system of this new permanent plant will be a modification of the present installation, employing multiple units of the same filter box.

SEA-WATER SYSTEM AT THE POINT WHITNEY SHELLFISH LABORATORY

By C. E. Lindsay

State of Washington Department of Fisheries, Brinnon, Wash.

Abstract.—A description of the original sea-water system of the Point Whitney Shellfish Research Laboratory of the Washington State Department of Fisheries includes local hydrographic conditions, piping, storage, pumps, distribution facilities, automatic controls, and provisions for cleaning. Considerations of cost, toxicity, materials available, and dependability determined the design. Although many of the operational problems inherent in this type of installation were solved initially, additional ones came to light with operation of the system. Some of these were subsequently eliminated by modification of various components, and others simply borne as limitations of the system. Later additions to the storage facilities and piping utilized new nontoxic plastics to expand capacity and versatility and eliminate damage from boring and fouling organisms. The effect of green concrete on quality of water was recognized. Recommendations for improvements to the present system and for development of a new system are made.

The Point Whitney Shellfish Laboratory of the State of Washington Department of Fisheries is on the west shore of Dabob Bay in Hood Canal, about 60 miles by road from Seattle. At this point Dabob Bay is approximately 1½ miles wide, has a narrow intertidal zone, and is 600 feet deep in the middle. Extreme tidal range is 17 feet. Surface temperatures range from 6° C. in winter to 21° C. in summer, and salinity ranges from 30 parts per thousand in winter to 24 parts per thousand in summer. Details of the hydrography of Dabob Bay are described by Westley (1956). Construction of the laboratory was completed in 1953, using an initial appropriation of $89,000. The sea-water system was incorporated into the building for use in conducting physiological experiments and bioassays and for live holding of oysters, clams, crabs, shrimps and other marine invertebrate animals. The system was relatively small, since the required uses did not include public aquarium displays or vertebrate fish holding facilities.

CONSTRUCTION AND OPERATION

Construction and operation of a sea-water system suitable for invertebrate animals has required the solution of many problems. The resultant system was a compromise between cost, toxicity, materials available, and dependability. The design was based on recommendations by fishery researchers on both the Atlantic and the Pacific coasts and our own previous experience with a smaller system at the Gig Harbor Laboratory.

Funds were insufficient to permit construction of duplicate continuous-pumping sea-water systems. Therefore a single-line system having foot valve, 4-inch main lines, main pump, standby pump, and 10,000-gallon wood storage tank was designed. Solutions to most of the problems peculiar to sea-water systems for invertebrates have been achieved through initial design and construction features, and by subsequent modifications to correct deficiencies and extend the system for additional uses. The problems and solutions we used are briefly described in the remainder of this report.

Toxicity of the system was reduced as much as possible by the use of Johns-Manville Transite pipe for those portions of the lines which were outside the laboratory building, extending from the lower end of the suction line into the building and from the building to the sea-water storage tank. Inside the building, main lines were of 4-inch threaded hard rubber with 2-inch hard-rubber distribution lines carrying sea water to the aquarium area, and half-inch hard-rubber lines to individual laboratory sinks. Originally the only metal in contact with sea water consisted of a cast-iron foot valve, three cast-iron elbows, a 60- by 32-inch steel plate covering the top of the suction screen box, six cast-iron flanges, and four threaded steel pipe adapters used to connect the pump to the suction box and main discharge lines. Several types of protective coatings have been used on metal parts exposed to sea water. In order of use they were: Nitrose, Atco carbon elastic black, Gaco, epoxy resin, and powdered polyethylene. None of these has provided complete protection, but in general epoxy resin and powdered polyethylene appear most satisfactory. A major source of metal contact was the steel suction-box cover, which was finally protected with a ½-inch-thick neoprene blanket used as a gasket between cover and sea water in the box. In time most of the cast-iron and steel flanges and adapters were replaced with threaded Uscolite pipe.

The main pump from the beginning was an Ace Hard Rubber Company 2½-inch WEFM pump driven by a 7½-horsepower single-phase electric motor. The standby pump was a 1-inch 1-horsepower Gardner-Denver cast-iron centrifugal pump. Because of cost as well as anticipated corrosion difficulties, valves were kept to a minimum. Originally only two 4-inch rubber-lined diaphragm valves were incorporated in the discharge lines, positioned so as to permit direct pumping without use of the storage tank, or to permit use of storage-tank water only and allow dewatering of intake, suction screen box, pump, and initial section of the main discharge line.

Protection of the pump from physical damage or clogging is a vital consideration. Damage from ice formation was prevented by burying intake pipes underground and incorporating the pump room inside the main laboratory building. Exclusion of bottom detritus or substrate material such as gravel has been achieved by plugging the end of a 13-foot length of Transite pipe and perforating this pipe with ¼-inch holes spaced on ¾-inch centers over the full length of the pipe. Except for occasional growth of seaweed or accumulation of heavy barnacle or mussel set on the interior of the pipe, this type of screened intake has been entirely adequate.

However, when using hard rubber as a pump liner, it is also essential to eliminate the abrasive damage caused by shells of barnacles, mussels, or oysters which have set on the interior of the pipe and may later become detached and sucked into the pump. To avoid this difficulty, a suction screen box was cast as an integral part of the building inside the pumproom, and adjacent to the seaward wall of the building. In plan view, the shape of the box is a trapezoid whose bases are 54 inches and 28 inches; altitude is 38 inches, and depth 36 inches. It has a volume of approximately 32 cubic feet. Two screen slots were cast into the concrete. The 4-inch suction line was cast into the box on the short base of the trapezoid, and two pump suction flanges provided on the long base. A 12 by 12 mesh per inch Saran plastic screen on wood frame was kept in one of the slots at all times and the top of the box sealed by a ½-inch steel plate and neoprene gasket, bolted down with 24 ¾-inch bolts set in a steel frame cast in the concrete (fig. 1). Elimination of most of the metal contact with sea water was finally achieved by placing a ½-inch-thick neoprene blanket between steel plate and suction box top. Holes for probe switch rod, primer pipe, and air cushion chamber were closed by threaded washers attached to black iron pipe nipples. In practice this device has proven somewhat troublesome to maintain, but has been valuable in providing combination screen and settling basin to minimize the amount of sand or other hard objects passing through the hard-rubber-lined pump. Certain design modifications of this type of suction box would eliminate features we have found unsatisfactory and still retain the basic advantages of the device.

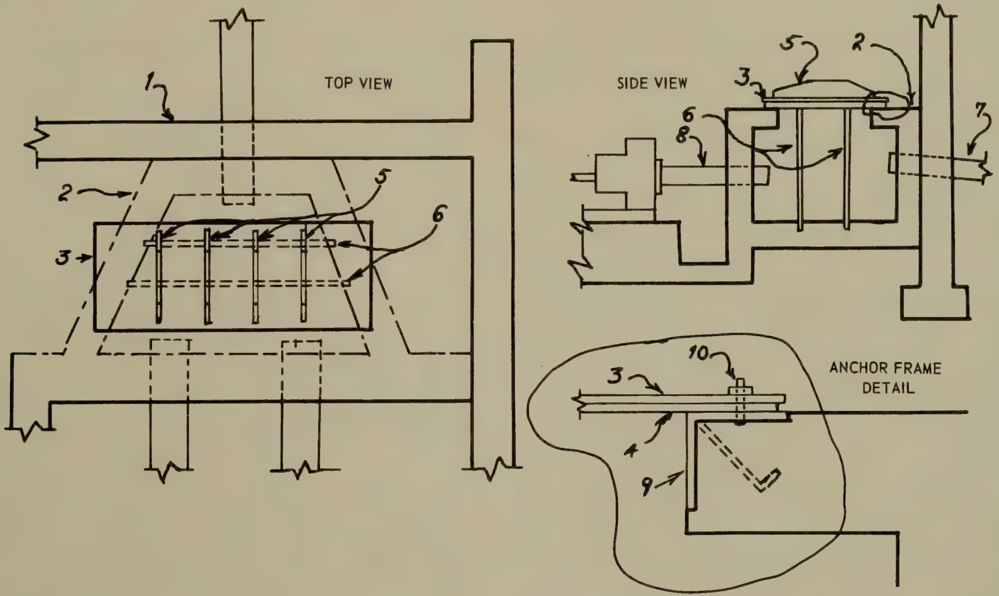

Figure 1.—Construction detail of the suction screen box at Point Whitney Shellfish Laboratory of the Washington State Department of Fisheries: *1*, building wall; *2*, suction-box wall; *3*, steel cover plate; *4*, gasket blanket; *5*, strong back; *6*, screens; *7*, 4-inch sea-water-intake line; *8*, 4-inch pump suction line; *9*, 4- by 4- by ⅜-inch angle iron; *10*, studpost.

The most important modification would be to change the relative position of box inlet and outlets so that pump suction is at least 12 inches below the bottom of the incoming sea-water suction line. At the same time the volume of water retained in the box above the pump suction should be nearly equal to the volume of the sea-water suction line lying above the level of half tide.

A source of difficulty has been maintenance of adequate vacuum while the system is on automatic operation, since porous spots in the concrete and poor bonding between anchoring ring and concrete sometimes allowed air to leak into the box. Box walls, therefore, must be of high and uniform density and should be concrete at least 10 inches thick. To reduce the area that must be fitted with removable gasket, the cover-plate opening should be made only large enough to allow insertion and removal of two screens. The cover-plate anchoring ring should be fabricated of 4- by 4-inch angle iron and should be embedded entirely in concrete, with only the steel hold-down bolts extending above the concrete surface. Plate hold-down bolts must be spaced at intervals no greater than 7 inches, and must be at least ¾-inch diameter to eliminate flexing of the plate and weakening of bolts by rust.

Pumping throughout the large tidal range creates a variable suction head as well as considerable variation in water temperature and salinity. In order to minimize these effects, the system was operated by intermittent pumping, which was as a rule done at any stage of tide higher than half-tide. This minimizes the danger of loss of prime of the pump, but does not completely eliminate it. Malfunction of the check valve regardless of the stage of tide is also a factor in loss of prime. Since funds were not available for an automatic vacuum system, we have used the fresh-water supply as a source of priming water. This is achieved by making direct connection between the main fresh-water line and the main sea-water line so that an adequate volume could be flowed directly into the pipe or built up in the storage tank to provide enough water for priming even when the check valve was stuck wide open. The pump control system is designed to be either automatic or manual. When lines were filled, repriming was accomplished by switching electrical controls from automatic to manual and allowing the pump to refill the storage tank.

In addition to the normal safety switches for protection of the pump motor in case of overload or low voltage, an interlocking safety switch and control system was designed. An ordinary mechanical float switch with styrofoam flotation was used in the main storage tank to prevent overfilling. A mercury switch, activated by air pressure and connected to an open-ended plastic pipe on the beach, was used to provide an adjustable tide-level switch. In operation, as the tide recedes below a predetermined level, this switch shuts off the pump so that pumping cannot occur, and as the tide rises, air pressure in the line reactivates the switch so pumping can occur on demand. To protect the hard-rubber-lined pump from being run without water, an electric probe switch in the suction screen box coupled to a relay provides positive protection against loss of prime and prevents automatic restarting of the pump following power outage. This loss-of-prime safety switch has not been entirely trouble free, but we have found in practice that as long as it is checked daily and cleaned occasionally, it provides adequate protection for the pump.

Sea-water storage facilities, as mentioned in the beginning, initially consisted of a single 10,000-gallon wood storage tank placed on a hill at an elevation approximately 6 feet above the roof of the laboratory building. When full, it provided a maximum head of about 15 feet. Since antifouling paints, or applications of toxic chemicals to prevent or remove boring organisms would also jeopardize other in-invertebrate animals, it was originally planned to treat the tank periodically with hot fresh water. However, the volume required to adequately treat the tank was so large (4,000 gal.) that it was impractical to use this approach. Partial protection was achieved by coating the bottom with high-melting-point roofing asphalt and the walls with bituminous water-tank paint. Later a polyethylene liner was installed to achieve better protection of the tank walls. In spite of these efforts, the walls became so riddled with teredos (*Bankia setacea*) that the tank had to be replaced after 7 years' operation.

A new 15,000-gallon concrete storage tank was built adjacent to the original wood storage tank. It was provided with a concrete cover, the floor was properly sloped for adequate drainage and flushing, and a direct fresh-water connection was installed. Since no substance which we were sure was not toxic to invertebrate larvae appeared to be practical for sealing green concrete, we allowed the tank to leach out naturally in sea water. It is particularly important to note that "green" concrete had significant toxicity to Pacific oyster larvae for more than 9 months after the tank was completed.

In the course of conducting a 3-year bioassay on adult Olympia and Pacific oysters, additional sea-water storage capacity was needed. To provide this at a reasonable cost, we erected nine 5,000-gallon unpainted Douglas-fir storage tanks, which were lined with 4-mil polyethylene sheet. This made a generally satisfactory liner which prevented attacks by marine borers and provided proven nontoxic water contact surfaces. We found that the 4-mil sheet, unless very carefully handled, was subject to abrasion or cutting. After 2 years, replacement was made with shaped 8-mil polyvinyl-chloride liners. These liners were found to be toxic. At writing, we are still trying to determine what component of the liners was the source of the toxicity. In previous tests, solid polyvinyl-chloride fittings were nontoxic. We have to assume that some type of plasticizer used in the bonding of the seams or manufacture of the film may be the toxic agent.

Overhead feed lines have always been difficult to maintain in trouble-free operation. In order to solve this problem at Point Whitney, and provide easy cleaning, the aquarium distribution lines were 2-inch hard rubber with T or cross fittings in place of elbows at each turn. Instead of placing potentially troublesome valves on the ends of the distribution lines, a 2-inch neoprene radiator hose was clamped to the end of a hard-rubber pipe nipple, and the flow was adjusted by means of a wooden pinch clamp. Water flowed into 8-inch-deep open wooden header troughs running the length of each room. The header trough has an advantage in that holes may be bored any place along the trough to provide flow through glass tubes of constant orifice for individual or groups of aquariums or trays, the only limitation in capacity being the size of the distribution line itself. Even though marine plywood was used, teredos (*Bankia*) riddled the header trough and lower aquarium trays. The trough was coated with hot roofing tar, and the trays were rebuilt with polyester resin coating. This has been satisfactory.

Clearing sea-water lines of marine fouling organisms is a potential problem in any area where surface waters are used. At Point Whitney, common fouling organisms are mussels, barnacles, oysters, tunicates, serpulid worms, and sea anemone. In addition to reducing the rate of water flow, they also compete with experimental animals for food, and contribute metabolites to the water. At Point Whitney, provision was originally made both for cleaning by hot water and for mechanical removal. Several sources of hot water were tried, including domestic furnace, domestic hot water, recirculation of specially heated water, and a portable steam cleaner unit of the type used in automotive repair shops. None of these supplied enough heat to kill all fouling organisms throughout the lines. We finally obtained a 400-gallon heavy steel tank, mounted this over a firebox, and used it to provide a charge of boiling water of sufficient volume to completely fill each separable unit of sea-water intake and discharge lines. It has been satisfactory and economical to use, since thorough cleaning about twice a year is adequate. However, owing to the difficulty of removing salt water from the lower end of the submerged suction line and replacing it with hot fresh water, we finally turned to the use of a mechanical pipe cleaner (Roto-rooter) which is used twice a year to ream out the line.

Three-quarter-inch flexible polyethylene pipe used in part of the system also became clogged with growing mussels. In time it became impossible to remove these mechanically or by hot-water treatment. We were forced to use concentrated hydrochloric acid in these lines to kill animals and partially dissolve their shells. Recovery of the hydrochloric acid has made this type of cleaning economical, and thorough flushing with sea water removed all traces of acid.

Shortly after the system was in operation, we found that the lower end of the sea-water line was being heavily pounded because of the sudden change in momentum of the water when pumping was stopped. A shock-absorbing effect was achieved by attaching 4-foot vertical 4-inch steel water pipes to the sea-water lines both ahead of and behind the pump. This cut down the water hammer, and no further damage from this cause has occurred.

In 1957, additions to the sea-water system were made which included wooden storage tanks mentioned before, and additional sea-water discharge lines to carry water to control, mixing, and delivery tanks for a long-range sulfite waste liquor bioassay. By this date, flexible polyethylene and semirigid Kralastic pipes and PVC fittings without plasticiser were readily available, and had been proven nontoxic in sea-water system use. We installed about 225 additional feet of 3-inch plastic sea-water line beyond the main storage tank, to an elevation approximately 20 feet higher than the previous maximum water level. Kralastic and PVC were joined with glue on the job without special equipment. Modifications or correction of errors were made simply by sawing the pipe off with a handsaw and inserting the correction with slipover couplings. This type of pipe has been impervious to fractures, climatic extremes, and chemicals.

The original water demand on the sea-water system at the time of initial construction ranged between 10,000 and 20,000 gallons of water per day. With increased use and extension of the system, demand increased to approximately 50,000 gallons per day. Throughout this period, the WEFM hard-rubber-lined pump originally described has been adequate to provide all the water required and still maintain intermittent pumping generally in the tidal ranges desired. It is anticipated that in our location this system could produce up to 100,000 gallons a day without enlargement.

We have found it essential to toxicity-bioassay, by means of marine invertebrate larvae, all of the component materials of the sea-water system, determine their degree of freedom from toxicity, and subsequently, bioassay quality of water supplied to experimental animals.

There are marked differences in growth, survival, and fatness of oysters held in the laboratory and those grown in the bay. We have found that oysters suspended near the surface do best, those grown on tidelands are poorer, and those in the sea-water storage tank are poorest. In each instance the groups of oysters tested had access to virtually unlimited quantities of water. Hydrographic data collected just beyond the intake shows stratification, during the period of highest water productivity, between surface waters and those normally tapped by the sea-water system. Chlorophyll values are also higher in the bay surface water than at either sea-water intake level or at point of entry into laboratory aquariums. These observations indicate that water quality could be improved by means of a floating intake line positioned so as to pump water from the surface layer where water productivity is highest. Several difficult physical problems must be solved before this method would be satisfactory for our location, and as yet this component has not been built.

CONCLUSIONS

The sea-water system at the Point Whitney laboratory has provided satisfactory service when operational factors of this location are considered. Preventive maintenance by skilled personnel is a critical factor in maintaining this or any other sea-water system in continuous year-round operation. It is clear to us that a dual system using present-day nontoxic plastics would probably be more dependable, lend itself more readily to preventive maintenance, and be definitely easier to keep free of fouling organisms, than our present system. Inasmuch as funds are not likely to be available for the complete rebuilding of the system, modifications of the present system must be carefully engineered to utilize existing equipment so far as possible.

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SEA-WATER SUPPLY SYSTEM IN A SHELLFISH-CULTURE LABORATORY

By P. R. Walne

Ministry of Agriculture, Fisheries, and Food, Conway, Wales

Abstract.—The sea-water supply system of a set of laboratories devoted to the culture of bivalve mollusks is described. The water is distributed from roof tanks to a tank room where it is heated and enriched with phytoplankton, and to the hatchery where it is filtered and sterilized. The equipment used for heating, filtering, and automatically enriching with algal cultures is discussed.

The sea-water system described in this paper is used in a laboratory engaged in investigations on the culture of larval and adult bivalve mollusks. It is built on a site adjacent to a set of concrete tanks constructed 50 years ago for mussel purification, and the pumping facilities of these tanks are also used to deliver sea water into the main laboratory storage tank. The site is on the estuary of the river Conway, and suitable-salinity water is obtainable only for a few hours at high tide. Apart from this, some storage is desirable to reduce the silt content of the water which is liable to be high during rough weather.

The use of metals has been avoided as far as possible in the construction of this system. The largest amount of metal is the iron main through which the water is first pumped. From the results obtained, this seems to be quite harmless, but the continual addition of rust to the water is a nuisance at times.

GENERAL LAYOUT

The water is pumped up from the estuary by a centrifugal pump with cast-iron body and impeller driven by a 30-horsepower electric induction motor and is delivered (for laboratory purposes) through 6-inch cast-iron pipes into a 20,000-gallon reinforced-concrete tank at 40,000 gallons per hour. From the main outside storage tank the water is pumped as required through a 2-inch polythene pipe into a set of three interconnected 100-gallon fiberglass tanks in the roof of the laboratory block. Fiberglass tanks were chosen because of their lightness and freedom from corrosion, and after tests had shown that oyster larvae could be satisfactorily cultured in them. The pump, which is close to the wall of the main storage tank, is a 2-inch pump (Mono Pumps Ltd., London) with a stainless-steel rotor and a rubber stator and is sited below the level of the water in the tank. This is important in a pump under automatic control, since it eliminates trouble due either to failure of the pump to prime or, if a foot valve is used, to leakage due to dirt or organisms growing on the valve. The pump is controlled by a float switch (Stuart Turner Ltd., Henley-on-Thames) in the laboratory roof tanks which are thus automatically kept nearly full of water. As there is no foot valve on the pump, the pipe empties to the level of the water in the storage tank when the pump stops, and this prevents the growth of sedentary organisms in the pipe.

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From the roof tanks the sea water is distributed to all rooms by standard polythene piping. Throughout the laboratories the flow is controlled by polythene valves (Saunders Valves Co. Ltd., Cwmbran, Newport, Monmouthshire). These valves, in which all parts in contact with the liquid are of plastic, are diaphragm valves which are closed by depressing the diaphragm until contact is made with a weir in the pipe line. In the laboratories, a supply is piped to plastic-lined pillar taps fitted with Saunders valves. Elsewhere the lines end in threaded Saunders valves to which various pipe lines for different apparatus can be attached as required.

HEATED SEA-WATER SYSTEM

This system feeds a supply of warmed sea water into the tank room to stimulate spawning in oysters in the winter months. It is the most elaborate part of the system and is therefore described in some detail.

The general layout is shown diagrammatically in figure 1. The water is heated in a fiberglass tank which stands alongside, and is connected to, the storage tanks in the roof. As its top is level with that of the storage tanks no level-control valves are required. The water is heated electrically by three 3-kilowatt immersion heaters which are coated with fused silica (Vitreosil, Thermal Syndicate Ltd., Wallsend-on-Tyne). These heaters, which stand vertically in the tank, are made so that the heating element is in the bottom 17 inches of the heater. Because this part of the heater must not be exposed to the air when it is on, the inlet and outlet of the tank are above this level, and in the event of a pump failure or excessive drawing off of water the heaters cannot become uncovered.

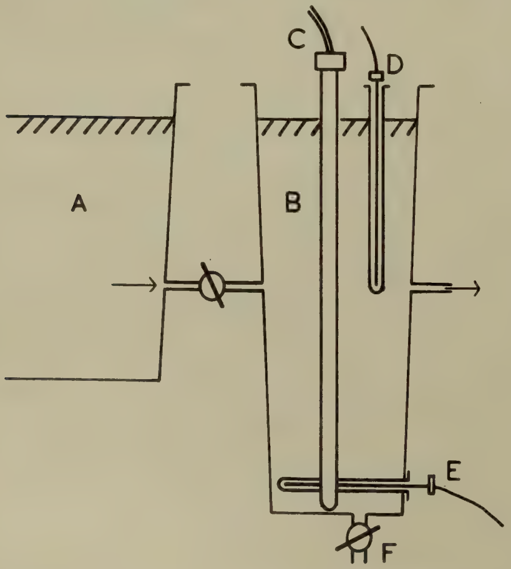


Figure 1.—System for warming flowing sea water: *A*, storage tank; *B*, warming tank; *C*, 3-kw. Vitreosil-covered immersion heater; *D*, thermostat in glass pocket controlling heater; *E*, safety thermostat; *F*, drain.

The heaters are controlled by bimetal thermostats (Sunvic T. S. 8, Associated Electrical Industries, London) inserted into a glass thermometer pocket fixed vertically in the tank. To improve heat transmission the pocket is filled with a heat transfer oil. The thermostat, which has a differential of 0.1° C., controls the heater through a hot-wire vacuum relay (Sunvic, Associated Electrical Industries, London). The basic heating is done by two heaters which are controlled by one thermostat, while the final control is given by a third heater controlled by its own thermostat. This allows the system to operate with 1, 2, or 3 heaters according to the requirements of the laboratory and the temperature of the incoming water, and avoids the disturbance to the laboratory electrical supply which might be caused by switching 9 kilowatts on and off. It is necessary to stir the water either mechanically or with compressed air; otherwise marked temperature stratification can occur.

As an additional safety measure, a thermostat in which the contacts close with rise of temperature is placed in a thermometer pocket fixed horizontally in the side of the tank near the bottom—and therefore never liable to be uncovered. This thermostat, set at about 60° C., operates through a contactor which, when energized from the thermostat, interrupts the current supply to all the heaters. The supply can only be manually restored.

This tank, with a volume of about 150 liters, produces about 200 liters per hour of sea water at 22° C. from water at 2° to 4° C., and although there are regular fluctuations in the temperature of the water, as it leaves the tank, due to the switching of the thermostat and the time taken for the heaters to warm up, these are damped in the 40-liter aquariums into which water is running at 10 liters per hour. A flow rate of 200 liters per hour raised through 20° C. is about two-thirds of the maximum possible with an input of 9 kilowatts. This is an adequate margin for cold weather or exceptionally large requirements. The degree of temperature control given by this system is shown in the thermograph record from one of the aquariums, reproduced in figure 2.

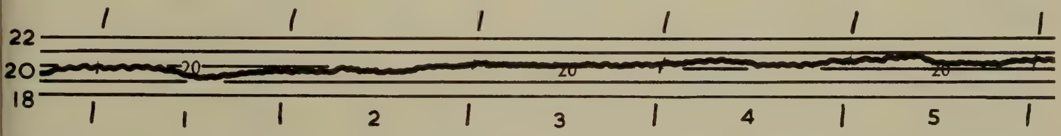


FIGURE 2.—Temperature record for 5 consecutive days in a 40-liter aquarium—flow rate about 10 liters an hour and the incoming temperature about 5° C. before heating.

The warmed sea water flows by gravity into the tankroom where, after it has been enriched with algal cultures, it is distributed by a polythene manifold to a series of aquariums. The water is enriched, usually with *Phaeodactylum* or *Dunaliella*, because in the winter months the natural flora of the water is at too low a level for filter-feeding animals which are being induced to grow and breed by raised temperatures.

The arrangement for continuously enriching the water is outlined in figure 3. The incoming sea water enters a wooden header tank through a hard-rubber ball valve (Dexine Rubber and Ebonite Ltd., Rochdale, Lancs.). This tank is at a higher level than the aquariums. Immediately above the header tank is a plastic tank which is filled daily with 20 liters of algal culture. This has to be stirred continuously to prevent settling of the algal cells. The culture leaves the tank through a tube set flush in the bottom to which is attached a rubber tube, which passes through a solenoid-controlled pinch clip (Londex Ltd., London), and then into the header tank. The culture, therefore, passes into the header tank when the solenoid is energized for a few seconds every 15 minutes—the length of time being adjusted so that the algal storage tank takes 24 hours to empty.

The timing device (fig. 4) has been constructed from a synchronous motor (Sangamo Weston Ltd., London), with a spindle speed of 1 revolution per hour. Four brass arms with adjustable points are attached to the spindle. A mercury tilt switch (I.A.C. Ltd., London) is arranged on a cradle with an adjustable arm so that each of the spindle arms in turn briefly touches the arm of the mercury switch and gives it sufficient tilt to allow the contacts to close. This opens the solenoid-controlled pinch clip for a period, the length of which is dependent on the adjustment of the contacts. The turbulence in the header tank provides sufficient mixing, and the following counts of the density of *Phaeodactylum* in the water entering an

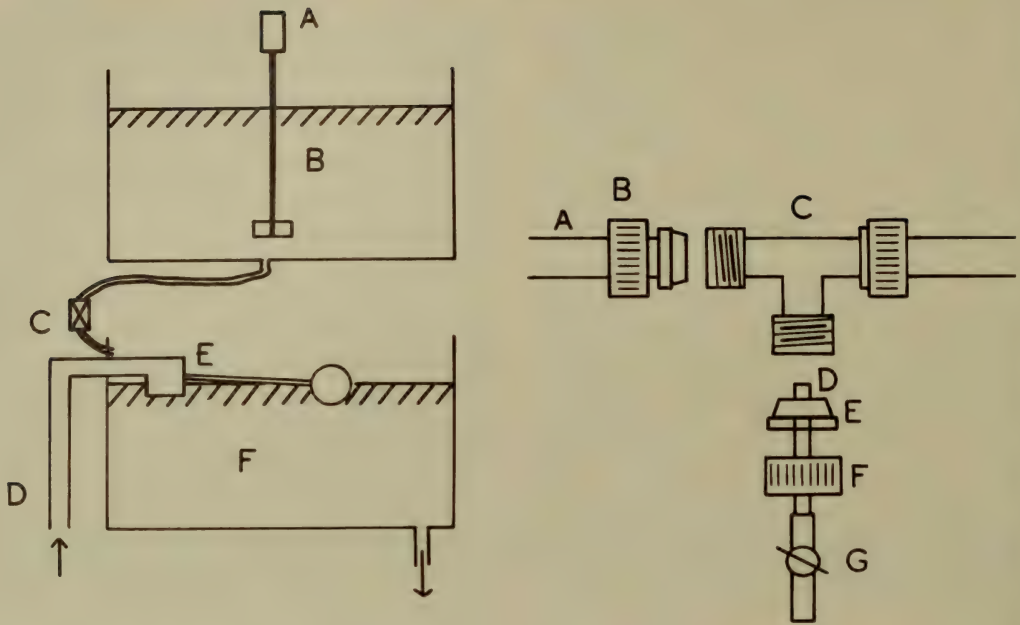


FIGURE 3.—Left—System for enriching the warm sea water with algal culture: *A*, stirrer; *B*, algal culture reservoir; *C*, solenoid-operated pinch clip; *D*, warm sea water supply; *E*, hard-rubber ball valve; *F*, header and mixing tank. Right—Detail of distribution manifold to aquariums: *A*, ½-inch polythene pipe; *B*, coupling nut; *C*, ½-inch polythene **T**-tube with screwed ends; *D*, glass tube; *E*, rubber stopper; *F*, coupling nut; *G*, Hoffman screw clip operating on a piece of rubber tubing.

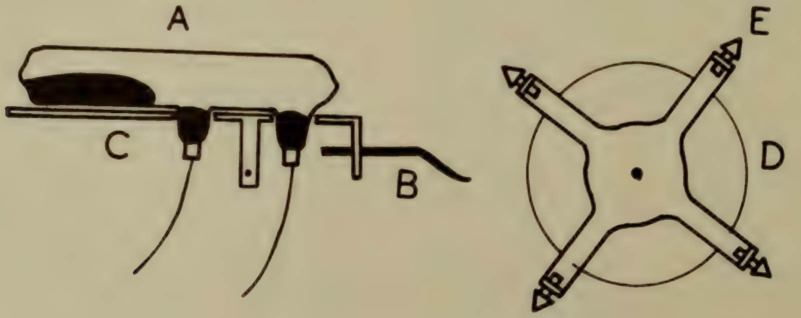


FIGURE 4.—Timing device for dosing algal culture into the sea-water system: *A*, mercury tilt switch; *B*, adjustable contact arm; *C*, brass cradle on a pivot; *D*, synchronous motor; *E*, 4 arms rotating at 1 r.p.h., each in turn making mechanical contact with *B*.

aquarium over a period of 15 minutes show the even amount of enrichment obtained:

Count of *Phaeodactylum* cells per cubic millimeter at—	
0 minutes	43
3 minutes	55
7 minutes	48
11 minutes	45
15 minutes	50

The enriched and heated water is distributed to the aquariums from a manifold made up of a series of **T**-tubes (fig. 3). The **T**-tubes and their interconnections are domestic cold-water polythene compression fittings (Plastronga, Yorkshire Imperial Metals Ltd.). As the flow rate is rather slow but continuous in this part of the system, various organisms grow rapidly in the pipes and it is necessary to be able to unscrew the system easily for cleaning. The descending arm of the T-tube is fitted with a rubber bung held in place by a coupling nut. A glass tube is passed through the bung and a piece of rubber tube with a screw clip is fitted on to control the supply to individual aquariums.

It is necessary to hold the tankroom at a constant temperature in order that the rate of cooling in the aquariums shall be constant. The rate of flow is about 10 liters per hour into a 40-liter aquarium and, as the system produces water at a constant temperature, variations in the cooling rate can have a considerable effect on a tank. It is such changes that cause the gradual variations in temperature recorded in figure 2, which are, however, less than those recorded in most natural environments. While equipping each aquarium with its own heater, thermostat, and stirrer gives more precise control, the cost is very much greater. The writer has found that such an installation does not give any better results and to have so much equipment in each tank is a nuisance.

HATCHERY

The sea water in the hatchery is used for the culture of oyster larvae and for this purpose it needs to be free from suspended material and substantially sterile.

The water is first filtered by pumping through a pressure filter. The pump is a ⅜-inch plastic pump with a neoprene impeller and stainless-steel shaft (Cleghorn, Waring & Co. Ltd., St. Albans, Herts.). The pressure filter (Aerox Ltd., Glasgow) contains a cylindrical ceramic filtering element 10 inches long by 2 inches in diameter. The body of the filter is of vulcanite. It is desirable to interpose a coarse filter before the ceramic filter, as the latter can clog rather rapidly, particularly if there is a lot of phytoplankton in the water. After filtration, the water is sterilized by ultraviolet light using a 44-watt low-pressure mercury discharge lamp (Hanovia Ltd., Slough, Bucks.). In this lamp the water circulates between an inner quartz jacket containing the lamp and an outer glass jacket. This pump and filter unit pass about 300 liters per hour, a flow rate at which the ultraviolet lamp is able to kill more than 99 percent of the bacteria. The water is then passed directly via ½-inch polythene piping and Saunders valves to the larval culture tanks. It is also advisable to keep the piping from the ultraviolet lamp to the tanks as short as possible, since bacterial contamination can occur from the wall of the pipe, and bacteria which multiply on the inside of the pipe form a nucleus for recontamination.

SUMMARY

An account is given of a sea-water supply system in a shellfish-culture laboratory. Provision is made for heating the water to 22° C. and for continuous enrichment with algal culture.

AN AERATING DEVICE FOR SALT WELL WATER

By Donald W. Strasburg, *Fishery Research Biologist*

Biological Laboratory, Bureau of Commercial Fisheries, Fish and Wildlife Service,
U.S. Department of the Interior, Honolulu, Hawaii

Abstract.—Salt well water appeared to be a suitable medium for captive oceanic skipjack tuna except that the available supply was devoid of dissolved oxygen. A model aerating device, whose characteristics could be varied, was used to determine the features of a definitive aerator. The aerator was a tower containing 16 perforated horizontal trays spaced 2 inches apart; the well water was pumped to the uppermost tray, from which it showered successively through those beneath, becoming 95 to 100 percent saturated with oxygen when it reached the bottom. Skipjack were later maintained in this water.

A desire to increase the efficiency of capturing oceanic skipjack (*Katsuwonus pelamis*) has led the Bureau of Commercial Fisheries Biological Laboratory at Honolulu into a study of skipjack behavior. In addition to a high-seas investigation, the study has a shore phase in which skipjack are confined in ponds under controlled conditions. Because earlier attempts to establish skipjack in ponds were unsuccessful (Tester, 1952), a large amount of preliminary planning was undertaken in order to avoid what were regarded as pitfalls in the capture, handling, and maintenance of these sensitive fish.

Of utmost importance was the need for an abundant supply of clean, circulating sea water, preferably water of an oceanic rather than a littoral character. The Laboratory's dock and pond facilities are located in Kewalo, a Honolulu district with three sources of salt water: Kewalo Basin, the Pacific Ocean, and salt ground water (fig. 1). Although Kewalo Basin is immediately adjacent to the Laboratory ponds, its water was eliminated from consideration because of a variable contamination from industrial and maritime sources, and also because the small size of its water body might result in a drastic salinity drop during heavy rains. In addition, Kewalo Basin water is rather turbid, a condition deemed unsuitable for ecological and observational reasons. The cost of pumping Pacific Ocean water across 300 to 700 yards of reef and shore seemed prohibitive; also tending to eliminate ocean water were similar, if lesser, problems of contamination and dilution.

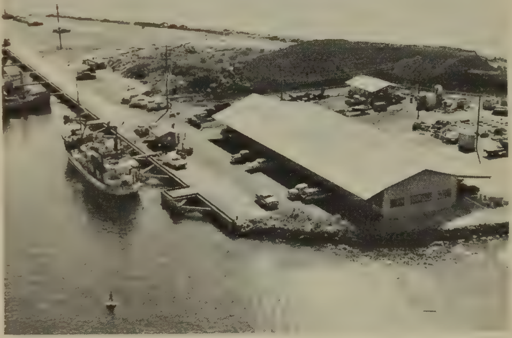


FIGURE 1.—Bureau of Commercial Fisheries dock facilities at Kewalo Basin, Honolulu, with the Pacific Ocean in the background.

Editor's note: Later developments of this system are described in the paper by Eugene L. Nakamura, which follows this one.

USE AND PROPERTIES OF WELL WATER

Because of the unsuitability of these sources, two salt-water wells were drilled at Kewalo in June 1958. The bores passed through hard-packed coral and sand to depths of 77 and 67.5 feet, of which the upper 42 feet were lined with 3½-inch casing (fig. 2). In November 1958, pumps were fitted to both wells, and studies were begun on the fitness of the water for maintaining various fish. Heavy pumping caused the shallower bore to become impacted with sand, but the 77-foot well has been in nearly continuous operation since that time. About 35 million gallons had been pumped in 20 months at rates varying from 30 to 45 gallons per minute. At these rates the drawdown of shaft water level was only 0.4 feet. Some properties of this water are listed in table 1, along with similar data for the Honolulu Aquarium well (3 miles from Kewalo) and oceanic water. The Kewalo and Aquarium samples were drawn on December 10, 1958, while the ocean water values were compiled from various sources, including the Honolulu Board of Water Supply. The analyses were performed by Y. F. Lee of the Honolulu Board of Water Supply, to whom I am indeed grateful.

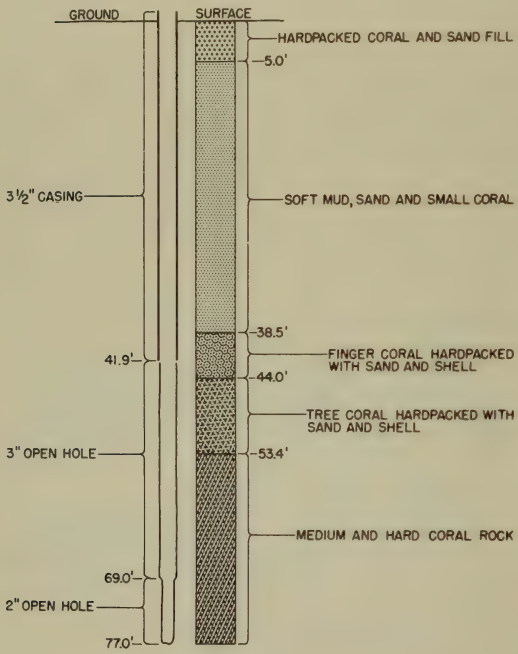

GROUND
SURFACE
HARDPACKED CORAL AND SAND FILL
-5.0'
3½" CASING
SOFT MUD, SAND AND SMALL CORAL
-38.5'
41.9'
FINGER CORAL HARDPACKED WITH SAND AND SHELL
-44.0'
TREE CORAL HARDPACKED WITH SAND AND SHELL
3" OPEN HOLE
-53.4'
MEDIUM AND HARD CORAL ROCK
69.0'
2" OPEN HOLE
77.0'

FIGURE 2.—Diagram of Kewalo well and surrounding stratigraphy.

The Kewalo and Aquarium samples differed from ocean water in chlorinity and pH, both items of concern to aquarists. The pH deficiency was easily remedied by heavy aeration of the incoming water, the pH value rising to 8.1 in about 40 minutes. The effects of brackish water on skipjack were unknown, but the slightly low salinity of the Kewalo water did not seem likely to be a serious problem. There remained to be determined, however, the salinity range to be expected following heavy rains and extensive percolation of pluvial water. Salinity and rainfall data are presented in figure 3, from which it is evident that rain had little diluting effect on the water supply.

Table 1.—*Some properties of Kewalo and Honolulu Aquarium well water and the nearby ocean*

[All values as mg./L. except pH]

	Kewalo	Aquarium	Ocean
pH	7.50	7.55	8.25
Silica	22	17	3
Calcium	400	438	405
Magnesium	1,211	1,251	1,315
Sodium	10,400	10,000	10,540
Potassium	365	375	400
Bicarbonate	144	137	----------
Sulfate	2,440	2,530	2,610
Chloride	17,800	18,400	19,150
Phosphate	0.10	0.10	0.20
Alkalinity[1]	118	112	115
Hardness[1]	5,965	6,225	6,400

[1] As $CaCO_3$ equivalent.

Conspicuously absent from table 1 are data on the dissolved oxygen in the well water. Winkler analyses of water direct from the pump gave oxygen values of 0.15 to 0.69 ml./L, the mean of 14 samples being 0.30 ml./L. This inadequacy was at first remedied by pumping the water over a perforated tray, from which it showered into a large tank containing reef fish. Al-

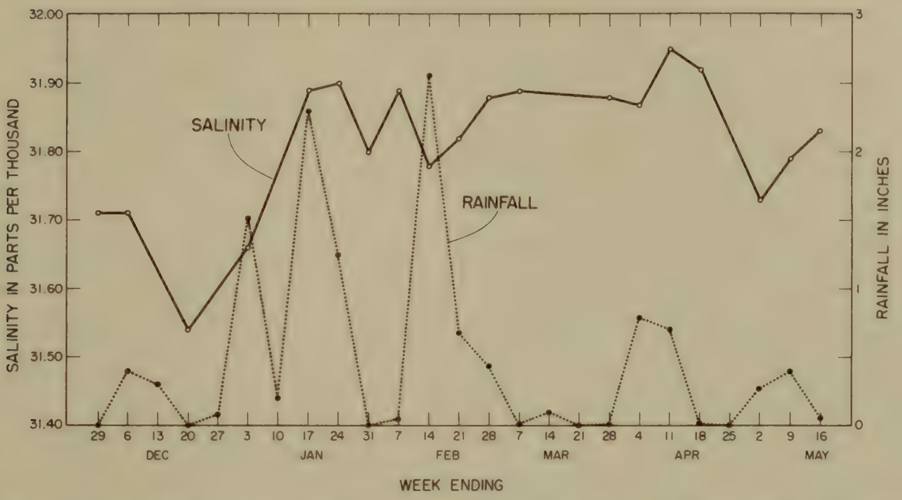


Figure 3.—Salinity of Kewalo well water and amount of rainfall at Federal Building, Honolulu, November 1958–May 1959. Rainfall data compiled by U.S. Weather Bureau.

though the oxygen content of this aerated water was as high as 2.34 ml./L, the fishes tested (*Abudefduf*, *Acanthurus*, *Arothron*, *Chaetodon*, *Parupeneus*, *Pervagor*, and *Zanclus*) did not thrive; in fact, many died within one or two days. In a closed-circuit control tank the oxygen level was maintained at 4.90 ml./L by continuous aeration with breaker stones, and here similar test fish did very well. It seemed obvious that an efficient aerating device must be an integral part of the pumping setup if circulating well water was to be employed.

AERATING DEVICES

A cursory survey of the literature plus correspondence with several aquarists revealed that little was known, or published, about the problem at hand. Specifically, a device was needed for bringing oxygen-poor water to the saturated state under high-flow conditions, and so constructed that oxygenation and circulation were concomitant features, eliminating any possibility of fish mortality from exposure to deoxygenated ground water. This requirement eliminated from consideration certain apparatus (Venturi systems, compressed gas liberators, and agitators) of great utility elsewhere (Norris et al., 1960). The field of choice was narrowed to sprays, cascades, and the like, where the influx of water per se results in aeration.

Plans for the aeration apparatus began to emerge when the physical conditions at Kewalo were considered. Beds of spraying fountains or shallow, baffled spillways were precluded by limited ground space, and the height of a tower from which water could fall was limited by the pumping pressure. Of various devices mentioned in the literature (Shaw, 1936; Betz Laboratories, 1957; McKelvey and Brooke, 1959), a compact block of stacked perforated trays seemed to be the most promising. Oxygen-free ground water would be pumped to the uppermost tray, from which it would shower successively through several lower trays, becoming saturated with oxygen just as it reached an aquarium below. The optima for tray spacing, number and size of tray perforations, rate of water flow, and tray number had yet to be determined.

EXPERIMENTAL AERATOR

To discover the interaction of these variables a model aeration device was constructed and a number of experiments were performed. Attention was focused on variations in dissolved oxygen resulting from different numbers and spacings of trays, while perforation size and number were held constant. Flow rate was necessarily low because of the miniature size of the equipment, but it was essentially constant at 182 to 193 milliliters per second (3 gallons per minute). Water temperature was also approximately constant, the range being 74.2° to 77.8° F. over the test period but with a fluctuation of less than 1 degree on any one day.

The apparatus assembled for a test is shown in figure 4; it consisted of the following parts: an open-faced wooden cabinet with a bottom drain and valve; an 11½-inch square wood and plastic inlet tray (actually an "under-gravel" filter) having 289 perforations and a brass nipple for attaching a hose; a number of 11½-inch square wooden trays having 144 perforations and no nipple; an ordinary ¾-inch plastic garden hose; a supply of metal C-clamps for positioning the trays in the cabinet; several sheets of thin vinyl plastic which were stretched across the face of the cabinet to prevent splashing; a short length of siphon tubing (not shown); and chemical equipment for determining dissolved oxygen by the Winkler method. In practice the flow rate, temperature, and amount of oxygen in the unaerated water were first measured, followed by the collection of samples from beneath varying numbers of equally spaced trays. The water samples were drawn from the bottom valve or by siphon; the drain was stoppered until 1 inch of water had accumulated in the cabinet's base, at which time a sample was taken.

Figure 5 shows the relations between tray spacing, tray number, and dissolved oxygen obtained from experiments with the model aerator. All oxygen values are shown in terms of percentage saturation for the particular salinity and temperature prevailing at the time of the test (the percentage saturation was obtained from data presented by Sverdrup et al., 1942, p. 188). The curves were fitted by eye to coincide with the maximum number of points. Not shown in the crowded upper left portion of the figure are data from tests using 10 to 15 trays; the curves from these tests lay between those for 9 and 16

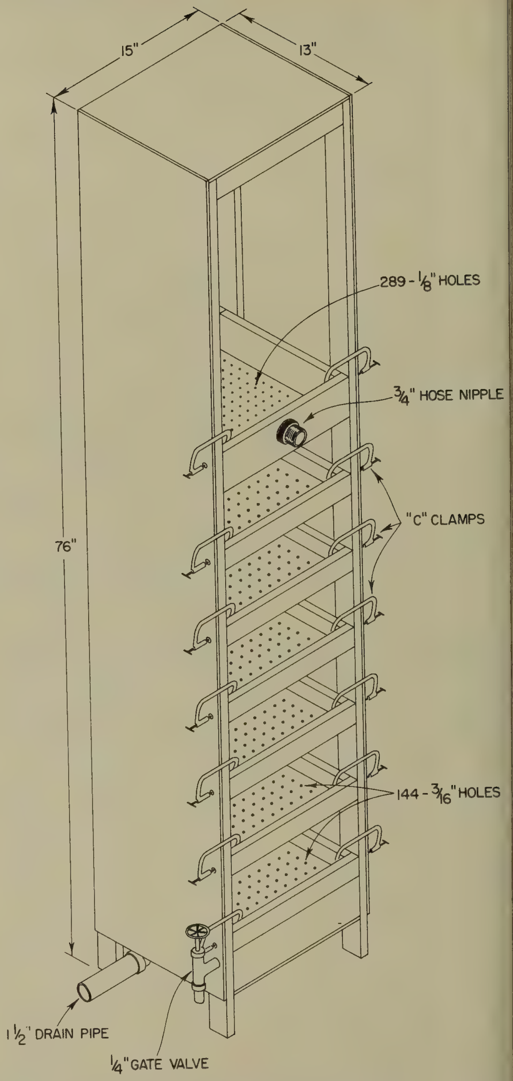
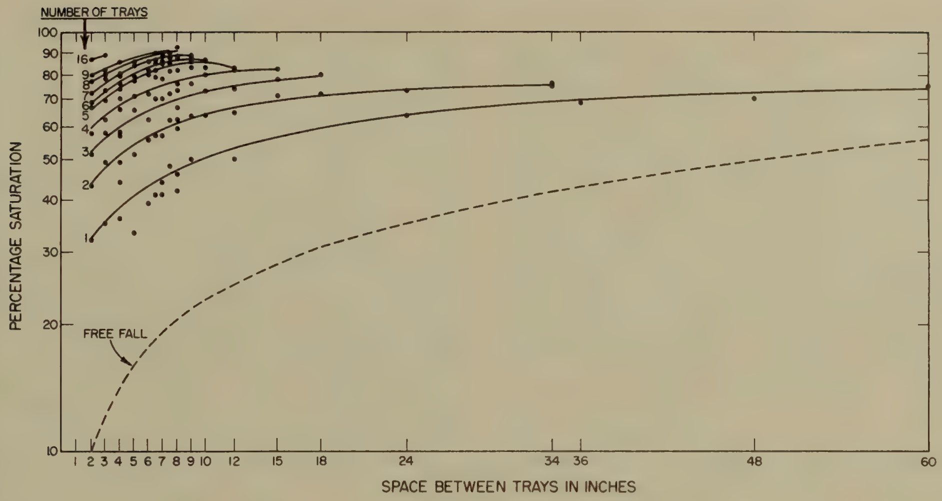


FIGURE 4.—Model aeration device used to determine effects of tray spacing and number on amount of dissolved oxygen in water. Water was supplied to the topmost tray by a hose attached to the nipple.



IGURE 5.—Relation between percentage oxygen saturation, tray number, and tray spacing. Broken line represents time/distance relation for a body in free fall.

ays. Also not evident is the oxygen con- ent of the incoming water; this ranged rom 0.15 to 0.69 ml./L, but was most ften 0.17 to 0.20 ml./L. The highest ıturation achieved with the model aerator as 93 percent; the highest obtained by rolonged bubbling of air through the ater was 96 percent.

Two aspects of figure 5 merit brief iscussion. The first is the shape of the ıturation curves: as is apparent, these ave the general conformation of the curve describing the distance traveled by a freely falling body. Saturation is a function of the amount of exposure to air, and this varies with drop size, velocity, and height of fall. The left portion of each saturation curve is flatter than the corresponding part of the free-fall curve, undoubtedly representing additional exposure to air because of splashing, flow over the tray surfaces, or a velocity decrease caused by air resistance. Also contributing to the shape of the curves are such factors as water

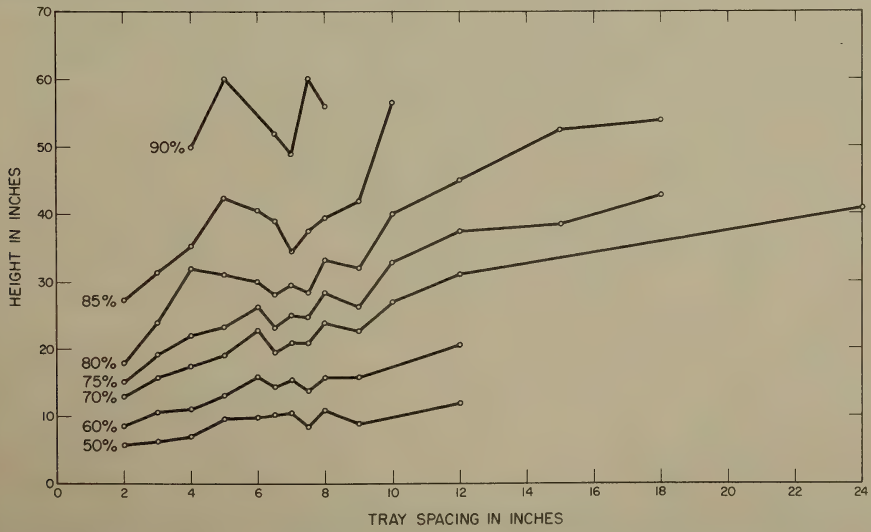


FIGURE 6.—Height of aerator needed to produce a given percentage of oxygen saturation.

films flowing down the cabinet walls, water drops falling cleanly through perforations aligned by chance, occasional stream-flow through a perforation, and the difficulty in adding oxygen to water as the saturation point is approached.

The second interesting feature of figure 5 is the relative importance of tray spacing versus number of trays. Although the same degree of saturation can be attained with either a few widely spaced trays or several closely spaced ones, there are practical considerations to be borne in mind when planning a definitive aerator. Wide tray spacing results in a tall aerator, requiring considerable pressure for pumping water to the top. Narrow tray spacing means a short device with numerous trays, the latter sometimes being expensive to construct and usually difficult to clean. Figure 6 shows the relative unimportance of tray spacing, for a con-

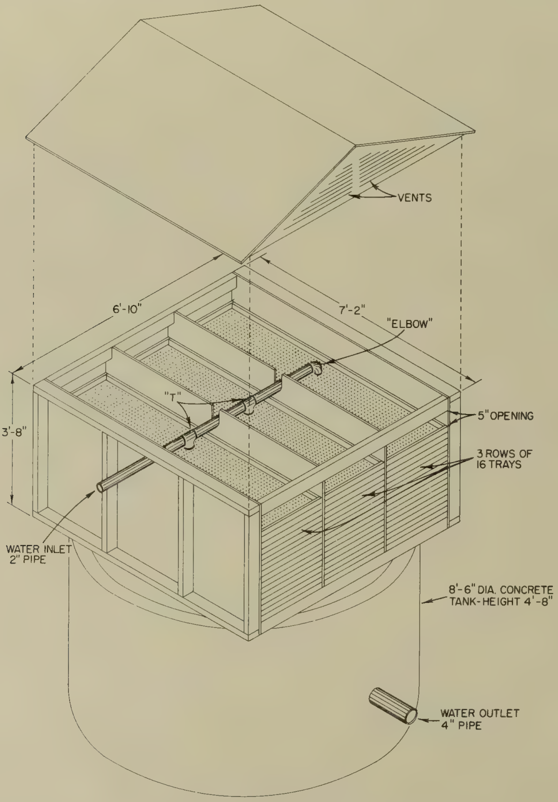


Figure 7.—Aerator developed for use with salt well water.

stant tower height, when only a low or moderate degree of saturation is desired. As higher degrees of saturation are attained, then the aerator becomes increasingly tall and spacing increasingly important.

DEFINITIVE AERATOR

Since it was desired to use highly saturated water as a medium for skipjack, some extrapolation was done from the upper curves of figures 5 and 6 in planning the definitive aerator. It was decided that 16 trays spaced at 2-inch intervals would provide near saturation with a moderate pressure requirement, and if these trays were perforated with $\frac{1}{8}$-inch holes at $\frac{1}{2}$-inch intervals there would be sufficient capacity to carry water at flow rates up to about 100 gallons per minute (and perhaps more). A diagram of the aerator ultimately built from these plans is shown in figure 7, from which the following features will be noted. The aerator is essentially a wooden box surmounting a concrete tank which receives the aerated water. The ends of the aerator are slotted for ingress of air, but light is excluded as much as possible to inhibit the growth of algae. Within the box are three rows of 16 long, narrow trays, which can be pulled out like drawers for cleaning or inspection. All construction is of wood except for the perforated tray bottoms, which are of pressed fibreboard doubly coated with an epoxy resin. The aerator has been in nearly continuous use for 18 months with only slight attention being required. Tray cleaning has not been necessary. Water passed through it ranged from 95 to 100 percent saturated with oxygen, and was successfully used for the maintenance of skipjack (see Nakamura, this collection).

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SALT WELL WATER FACILITIES AT THE BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL LABORATORY, HONOLULU

By **Eugene L. Nakamura**, *Fishery Biologist*

Biological Laboratory, Bureau of Commercial Fisheries, Fish and Wildlife Service,
U.S. Department of the Interior, Honolulu, Hawaii

Abstract.—A salt well water system was developed in Honolulu to maintain skipjack tuna (*Katsuwonus pelamis*) in captivity. Problems encountered with the system included accumulation of rust from the cast-iron casing in the well, inability of the well to provide sufficient water for additional fish pools, inaccessibility of the aeration trays for cleaning, necessity of frequent cleanings of the fish pool due to algal growth, and inability to observe the fish closely because of wind ripples. These problems were remedied by drilling a larger well with an asbestos-cement casing, by building a new aerator with trays spaced farther apart and easily accessible, and by enclosing the new pool in a building.

A salt-water system was developed at the U.S. Bureau of Commercial Fisheries Laboratory's dock site in Honolulu in 1958 to maintain skipjack (*Katsuwonus pelamis*), a pelagic tuna, in captivity. Salt water from a well was used because of its freedom from sediments, pollution, dilution by rains, and fouling organisms (barnacles, mollusks, annelids, etc.). The system comprised a well, a pump, an aerator, a sump on which the aerator rested, and a fish pool (fig. 1). Polyvinyl-chloride pipes were used. The well, properties of the water, and the development of a suitable aerating device have been described in detail by Strasburg [preceding article in this collection]. While skipjack were being kept in the pool (Nakamura, 1960), certain shortcomings of this original system became apparent. These problems and their remedies are described in this report.

The casing of the well was a 3½-inch cast-iron pipe. Rust from this casing was deposited within the plastic pipes and on the perforated trays of the aerator. A layer of rust up to a millimeter thick lined the pipes after 18 months of use. Although rust was believed not to be harmful to the fish, it was undesirable because it reduced the bores of the pipes and the perforations in the trays and indicated deterioration of the well casing.

The limitations of the well's capacity became apparent when over 100 gallons per minute were pumped, for the water level in the well then dropped below the foot valve, which was 6 feet below the surface. This inadequate flow precluded the installation of additional fish pools.

It thus became apparent that a new well was required with a noncorrosive and nontoxic casing and of a capacity which should accommodate planned expansion.

The aerator was originally constructed so that the trays could be removed for cleaning. After about a year and a half of intermittent use, however, warping of the trays and swelling of the wood separating the trays made removal impos-

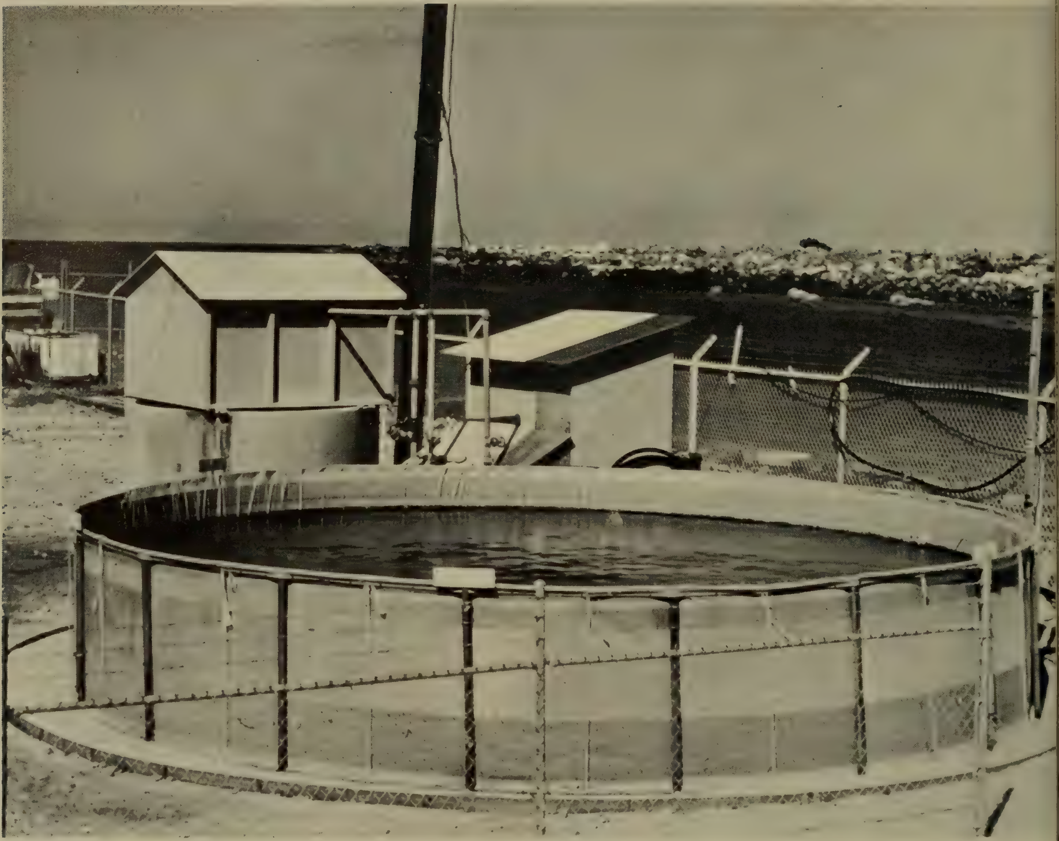

FIGURE 1.—Skipjack-holding pool, 23 feet in diameter and 4 feet deep. Behind the pool on the left is the aerator resting on the sump, and on the right is the pumphouse. The well, which cannot be seen, is between the pool and pumphouse.

sible. Since the trays were set 2 inches apart, the area between them was inaccessible for cleaning. Cleaning was desirable only because of the deposition of rust and not because of algal growth, which was inhibited by the enclosed situation of the trays within the aerator.

The aerator efficiently oxygenated the water (over 95 percent saturation), but did not adequately rid the water of carbon dioxide components. Excess carbon dioxide in the water caused the pH to be lower than that of normal sea water. The pH was 7.4–7.6 as compared with 8.1–8.3 in the open ocean (Sverdrup, Johnson, and Fleming, 1942). By rerouting the water through the aerator several times, the pH could be raised to 8.1 in 40 minutes, but this recycling was not practical.

In order to remedy these undesirable features of the aerator, plans for a replacement were made. The trays were to be spaced to permit cleaning. Better ventilation throughout the aerator was to be provided. Smaller but more numerous perforations were to be made in the trays so that the elimination of excess carbon dioxide would be improved.

The necessity of enclosing the pool in a building became apparent for two reasons First, the entrance of direct sunlight into the pool could be controlled to reduce or eliminate algae. In the outdoor pool, algae grew rapidly, and frequent scrubbings were required. Also, strands of a diatom (*Melosira* sp.) which broke off from the sides of the pool caused the water to become turbid. Second, the wind over the pool could also be reduced or eliminated. Wind ripples prevented clear observations through the water surface of the outdoor pool.

In August 1961 a new system was set into operation (fig. 2). The well is 73 feet deep, cased with a 12-inch asbestos-cement pipe down to 42 feet. The depth of the well was determined by the depth at which 1,000 gallons of water per minute could be provided. The depth of the casing was determined by the depth at which hard coral rock was reached. Water is presently being pumped at 82 gallons per minute, but the well is capable of providing water at 1,000 gallons per minute with a drawdown of 6 inches. A centrifugal pump with a cast-iron housing and a steel impeller is being used because this type of pump happened to be readily available. Thus far, its performance has been satisfactory.

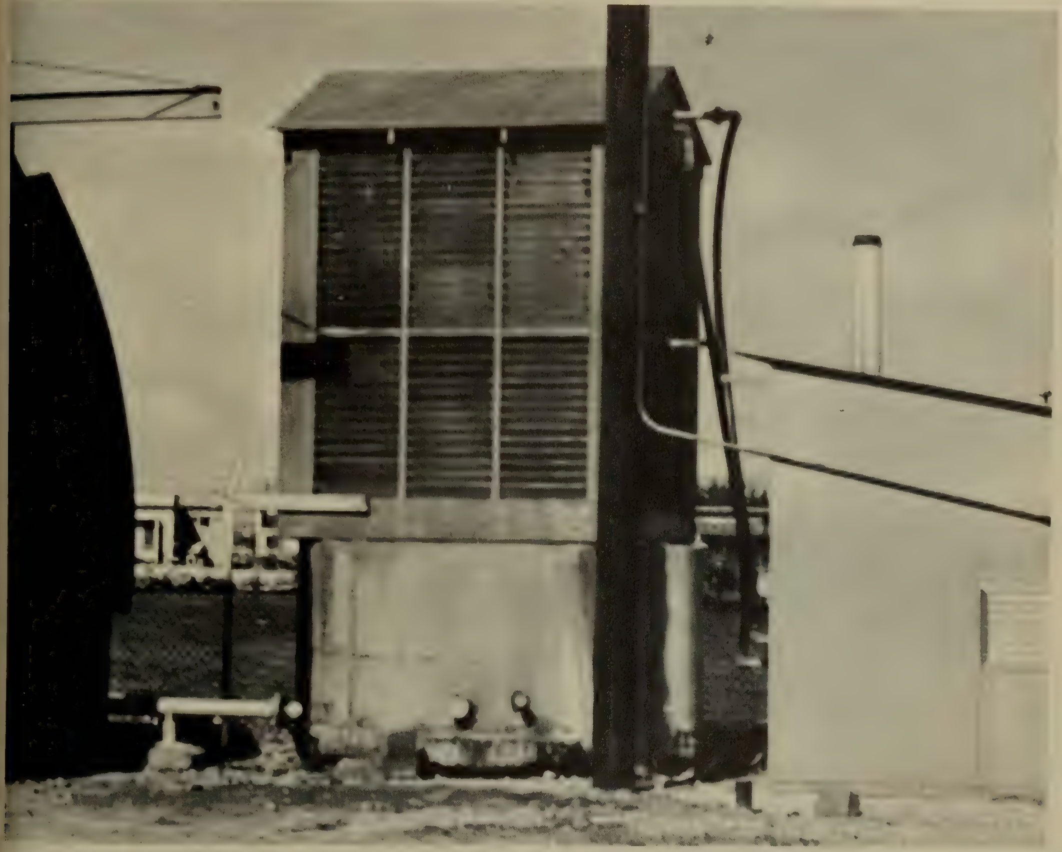

FIGURE 2.—Present salt-water system. The pumps and well are housed in the building at the right. The aerator, in the center, is resting atop the concrete sump. The fish pool is housed in the quonset hut on the left.

The aerator is 6 feet wide, 7 feet 9 inches long, and 10 feet high at its apex. All four sides have louvers. The two ends are hinged so that they may be opened for cleaning. It houses 16 aeration trays spaced 6 inches apart. The trays are made of laminated plastic, measure $1/10$ by 72 by 51 inches, and are perforated by $1/8$-inch holes spaced 1 inch apart. Water sprays onto the top tray from $1/8$-inch holes in the sides of the 2-inch inlet pipe.

The concrete fish pool is enclosed in a quonset hut. Water flows by gravity from the sump into the pool.

This new system appears to be satisfactory. Rust is no longer a problem. The pH has been raised to 7.8–8.0. The trays are easily accessible by opening the ends of the aerator. There is practically no algal growth in the pool, and the observation through the water surface is excellent.

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SEA WATER FROM GROUND SOURCES

By John R. Clark and Ronald Eisler

Sandy Hook Marine Laboratory, Bureau of Sport Fisheries and Wildlife, Fish and Wildlife Service, U.S. Department of the Interior, Highlands, N.J.

Abstract.—Efforts to obtain sea water from a ground-water source at the Sandy Hook Marine Laboratory are described. The basic theory of occurrence of fresh and salt water in coastal sand and gravel deposits is discussed and is confirmed by the results of a test well drilled at Sandy Hook. A hypothesis is advanced to explain both an unexpected reversal of salinity gradient and contamination by ferrous hydroxide. Although plans to maintain a continuous flow were frustrated, the water, after settling (to remove iron precipitate) has been used successfully in rearing 51 species of fishes and some invertebrates.

Many problems typically encountered in supplying large volumes of sea water to seaside laboratories can be solved by using a shallow ground-water source. The use of ground-water supplies involves a unique complex of problems, however, which must be anticipated before deciding in favor of a well system.

A major advantage of a ground-water supply is the elimination of barnacles, mussels, and other attaching organisms. These animals, which foul supply pipes and fittings, have no access to ground water. Also, sea water from a well is free from particulate contaminating material such as sand, silt, and detritus. Thus, filtration may be eliminated, and the design and construction as well as maintenance of the supply system may be simplified.

Another advantage of a ground-water supply is that physical characteristics of ground water remain relatively stable. Rapid fluctuations in salinity induced by tidal forces, which are so typical of estuarine or shallow bay waters, do not affect ground-water sources. The temperature of ground water is a function of average prevailing air temperature and, to a depth of 50 feet, is nearly constant for any location, varying a maximum of 1 degree annually. Therefore, ground-water temperature can be reliably predicted from air-temperature data (Collins, 1925).

A further advantage in using a ground-water source is that no special intake or pump support structures are required as for a direct sea-water source. These structures are often expensive and in many places are subject to damage by storms or, in northern locations, to damage by ice in winter. A well often may be located much nearer the laboratory than the inlet of a direct intake line, thus reducing cost and complications involved with lengthy transport lines.

We wish to express our appreciation to the U.S. Geological Survey (Trenton District Office) for their most helpful counsel and for arranging the analysis of our well water.

DESIGNING THE SYSTEM

This paper deals with problems encountered in obtaining sea water from subsurface sands and gravels at Sandy Hook, N.J. The decision to use a ground-water source for our sea-water supply was derived from a combination of factors involving geologic characteristics of the area, location of the laboratory, storm effects peculiar to Sandy Hook Bay, the small amount of funds available, and the anticipated requirements of our experimental aquarium.

The Sandy Hook Marine Laboratory is located 160 feet east of the easterly shore of Sandy Hook Bay (fig. 1). The Sandy Hook peninsula is a post-Pleistocene development that resulted from deposition of sand and gravel by northerly long-shore currents which are interrupted by the outflow from New York Harbor and Raritan and Sandy Hook Bays. The average depth of waters surrounding Sandy Hook is about 20 feet. This permeable sand and gravel deposit hydraulically connected to the bay appeared to provide an excellent source of good quality sea water. A well supplying a continuous flow system could be located at the edge of the Bay near the source of the water and yet be only a short distance from the Laboratory. The plan consisted of the following:

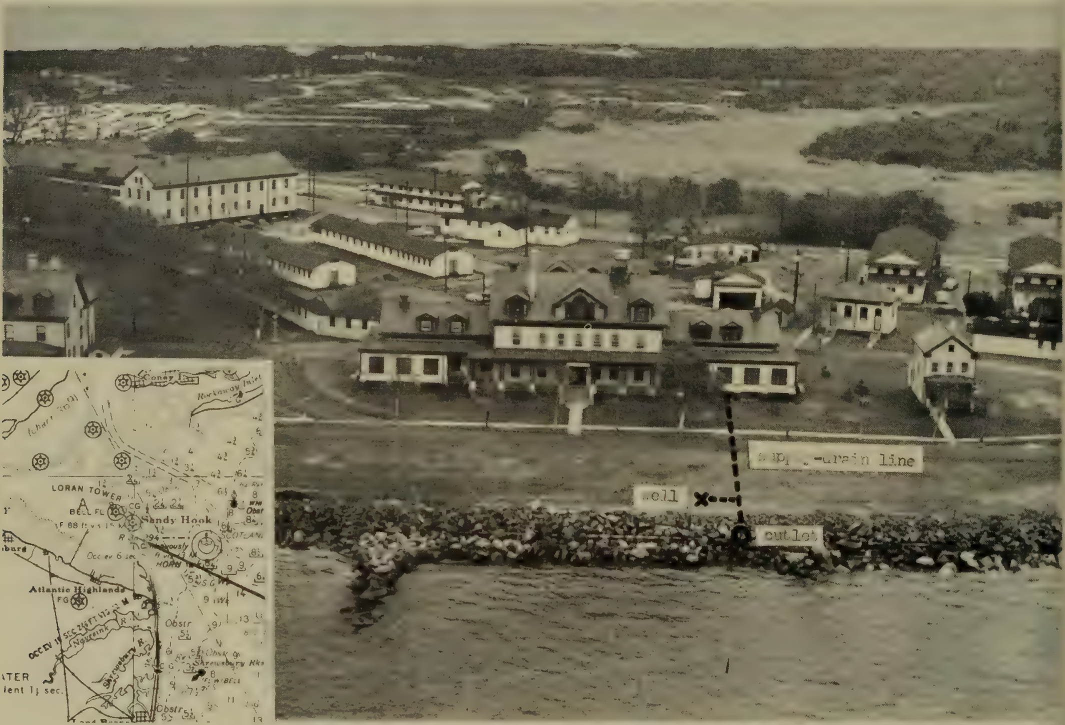

Figure 1.—Sandy Hook Marine Laboratory; Sandy Hook Bay in foreground, Atlantic Ocean in background.

1. A 4-inch-diameter polyvinyl-chloride (PVC) wellpipe fitted with a 4-foot-long PVC slotted intake screen (slots 0.014 inch in width) was to be inserted into a 6-inch-diameter (driven) iron housing and water-jetted to the minimum depth necessary to obtain sea water of full bay-strength salinity.

2. A nonmetallic submersible pump capable of a minimum pumping capacity of 20 gallons per minute was to be inserted into the 4-inch PVC pipe to maintain a continuous flow of sea water to the aquarium.

3. A 1¼-inch-diameter PVC transmission line to carry the water underground to the basement laboratory was to be run inside a 6-inch-diameter cast-iron housing.

4. A 3-inch-diameter PVC gravity-flow return line was to be run within the 6-inch iron pipe, the iron pipe to serve as protection for the re-

turn line and as an emergency overflow drain from the aquarium.

This plan for installing the well was achieved successfully, and except for a troublesome problem which was not anticipated—the presence of ferrous iron in the water source—would have adequately supplied our needs. This problem will be discussed in detail later.

THEORY OF SALT-WATER OCCURRENCE

In attempting to locate salt water, it was necessary to drill much deeper than had been anticipated. A series of test wells showed that, even at the very shore, with water level at 11 feet below ground surface, it was necessary to bore deeper than 40 feet to locate water of acceptable salinity. A similar situation is documented by Barksdale, Sundstrom, and Brunstein (1936) for ground-water supplies of the Atlantic City, N.J., region. Since this paper is difficult to obtain, relevant portions are given here.

> The problem of obtaining fresh water from sands that are exposed for a part of their extent to the waters of the ocean has been studied in many parts of the world. The earliest scientific work on this problem was done in Europe, where the basic principles were first pointed out in 1887 by Badon Ghyben,[1] a Dutch captain of engineers, and in 1900 by Herzberg,[2] who appears to have had no knowledge of the earlier work. The basic principles that govern the relation of salt water to fresh water in a water-bearing sand have now been fairly well established. They are discussed by Brown in papers published in 1922 [3] and 1925.[4]
>
> At the contact between the fresh and salt waters the zone of diffusion is surprisingly narrow. In Holland, Pennink [5] found a range of salinity from 100 to 15,000 parts per million of chloride in distances varying from 60 to 100 feet. In the present investigation ranges from 800 to 8,000 parts per million and from 1,900 to 7,300 parts per million were observed in 4 feet of depth.
>
> Salt water is heavier than fresh water and tends to fill the lower parts of a formation. The fresh water in the sand floats on the salt water much as ice floats on water, with most of its volume submerged. The position of the contact is determined by the head of the fresh water above mean sea level and by the relative specific gravities of the two waters. This is the principle developed by Badon Ghyben and Herzberg.
>
> This theory is illustrated in figure [2, A and B]. Figure [2A] shows a simple U-tube with both ends open to the air. The two legs of the tube are filled with two liquids of different specific gravities. The liquids in the tube will come to rest in such a way that the pressure at the bottom of one leg is exactly equal to and balanced by that at the bottom of the other leg. The surface of the lighter liquid will, therefore, necessarily stand higher than that of the heavier liquid. Furthermore, as the heavier liquid fills the lower part of the tube in both legs up to the level of the contact between the liquids, the pressure at this level is equal in both legs, and the heights of the two columns of liquid above the level of the contact are inversely proportional to the specific gravities of the liquids.
>
> In a small island or narrow peninsula composed entirely of permeable sand and surrounded by sea water, this same balance of pressure occurs between sea water and the lighter fresh water. Figure [2B] represents a cross section of such an island and shows that the salt water not only fills the sand around the island but also extends entirely under it below the lens-shaped body of fresh water. In such an island, the resistance of the sand to the flow of water causes the fresh water from rainfall to build up a head above sea level sufficient to cause it to flow out into the ocean at the shores of the island. It also prevents the mixing of the salt and fresh waters in the sand below sea level by wave action. As the sand is permeable in all directions, the fresh-water head will cause a downward flow of fresh water until it fills the sand to a depth at which its head is balanced by the head of the salt water. When equilibrium has thus been

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[1] Badon Ghyben, W. Nota in verband met de voorgenomen put boring nabij Amsterdam. K. Inst. Ing. Tijdschr, 1888–89, p. 21. The Hague, 1889.

[2] Herzberg, Baurat. Die Wasserversorgung einiger Nordseebader. Jour. Gasbeleuchtung und Wasserversorgung, Jahrg. 44. Munich, 1901.

[3] Brown, J. S. Relation of sea water to ground water along coasts. American Jour. Sci. 5th ser., vol. 4, pp. 274–294. 1922.

[4] Brown, J. S. A study of coastal ground water with special reference to Connecticut. U.S. Geological Survey, Water-supply Paper 537. 1925.

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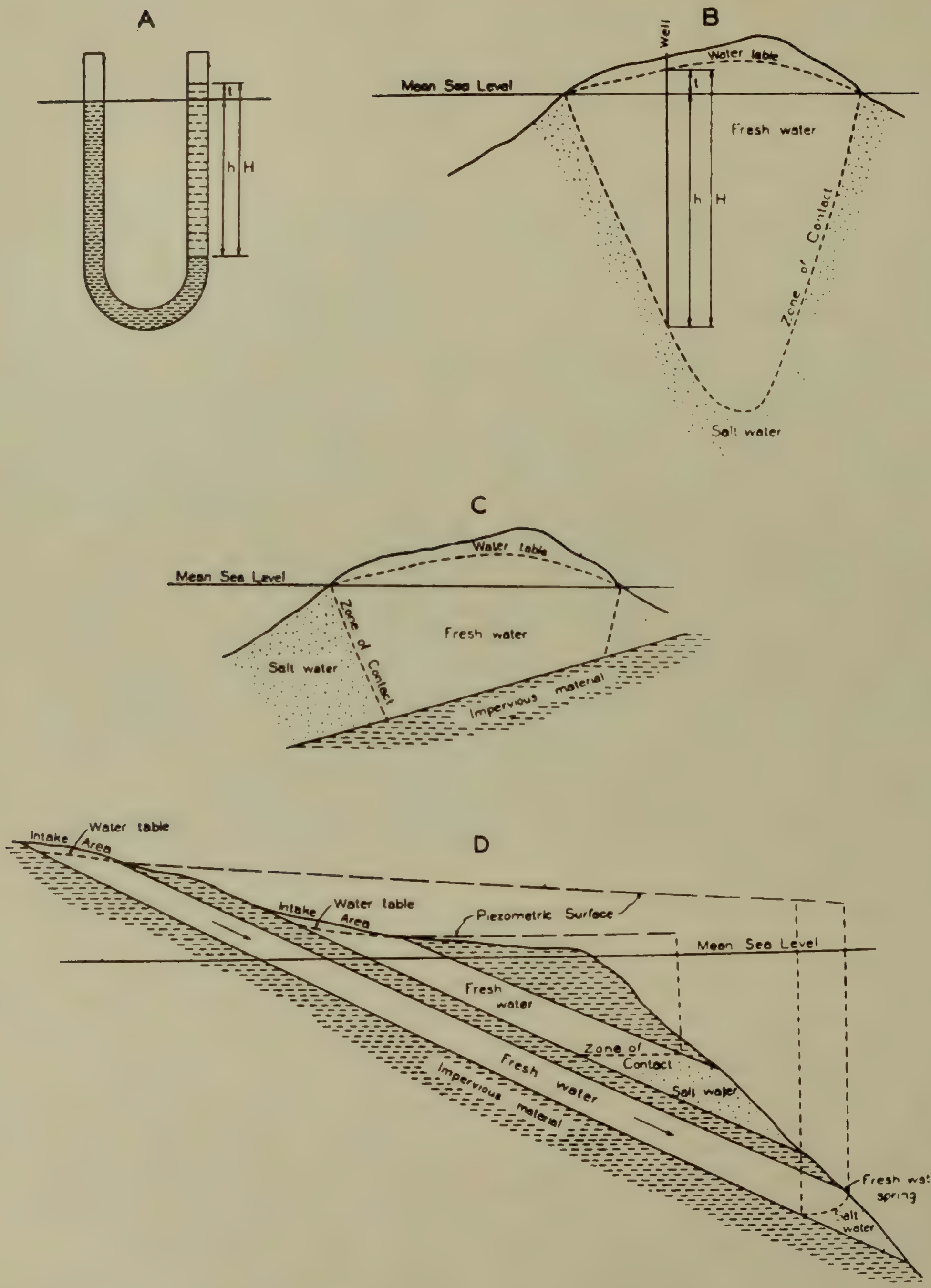

FIGURE 2.—Relation between fresh water and salt water in water-bearing sands when not disturbed by pumping: *A*, U-tube showing balance between two liquids of different specific gravities; *B*, section through an island composed entirely of sand and surrounded by salt water; *C*, section through a sand island underlain by impervious material; *D*, section through a coastal plain composed of alternate layers of pervious and impervious materials. (From Barksdale, Sundstrom & Brunstein, 1936—Supplementary Report on the Ground-Water Supplies of the Atlantic City Region; fig. 5, p. 26.)

reached, the depth of fresh water below sea level at any point on the island will be proportional to the fresh-water head above sea level at that point and the ratio between the depth and head of the fresh water will depend upon the relation between the specific gravities of the fresh and salt waters.

The following explanation, by Brown (1925, p. 16), of the relation between salt water and fresh water under a small sand island is applicable both to figure [2A] and figure [2B]:

Let H=total thickness of fresh water.
h=depth of fresh water below sea level.
t=height of fresh water above mean sea level.

Then $H=h+t$

But the column of fresh water H must be balanced by a column of salt water h in order to maintain equilibrium. Wherefore, if g is the specific gravity of sea water and the specific gravity of fresh ground water is assumed to be 1,

$$H=h+t=hg$$

$$\text{whence } h=\frac{t}{g-1}$$

In any case $g-1$ will be the difference in specific gravity between fresh water and the salt water.

If it is assumed that the specific gravity of sea water is 1.025, which is about an average figure, then $h=40t$. In other words, for every foot that the fresh water stands above sea level, it extends 40 feet below sea level. This ratio is so extreme that it is not practicable to show it in the various parts of figure [2]. For convenience, therefore, the first three parts of this figure have been drawn with a ratio of 1 to 10 between the head and depth of the fresh water. This would be the true condition if the specific gravity of the sea water were 1.100 instead of about 1.025. The fourth part of this figure is drawn with a ratio of 1 to 5 between the head and depth of the fresh water and represents an imaginary specific gravity of sea water of 1.200. The general relation between fresh and salt water shown by these diagrams is not affected in the least by this assumption of a specific gravity of sea water greater than the range that occurs in nature. The specific gravity of sea water varies from place to place, so that the figure of 1.025 used in the example above is only an approximate average.

In nature a body of land composed entirely of permeable material to any great depth is rare. The occurrence of beds or layers of impermeable material does not change the basic principles just discussed, but it does modify their application. If the island shown in figure [2B] were underlain by clay or bedrock that reached a level above the bottom of the fresh-water body, conditions such as those shown in figure [2C] would occur. Along the coast the position of the contact would be determined by the head of the fresh water, just as in an island composed entirely of sand, but under the center of the island fresh water would extend all the way down to the impermeable layer and would not be in direct contact with salt water.

The modification of conditions by impermeable formations is even more marked on the coasts of larger bodies of land, where water-bearing sands may lie under and between as well as above layers of impermeable material and may slope upward to remote intake areas well above sea level. Along such a coast the conditions in a permeable sand underlain by impermeable material would be similar to those in the sand island underlain by impermeable material, except that the fresh water would be in contact with salt water only on the side exposed to the ocean.

Figure [2D] shows two conditions which occur in water-bearing sands confined between layers of impermeable material. This diagram differs essentially from the others in that it shows the conditions that occur when the fresh-water in the sand is under artesian head rather than under water-table conditions. In the upper sand in this diagram, the salt water and fresh water are in balance, just as in the preceding examples. Salt water fills the lower part of this sand and fresh water fills the upper part of it. The position of the contact is determined by the head of the fresh water, which in turn is determined by the elevation of the intake area. The similarity between the conditions in this sand and those in the U-tube in figure [2A] is easily apparent.

In an artesian sand, the water is prevented from rising to the surface by the overlying impermeable bed. It is under a head that would cause it to rise in a well to a level above the bottom of the confining bed. The imaginary surface that would pass through the surface of the water in a well drilled to the sand at any point throughout its extent is called the "piezometric surface." The piezometric surface is therefore a pressure-indicating surface, and its elevation at any point indicates the head on the water in the sand at that point. At the intake area of the sand it merges into the water table which, though not imaginary, might be considered a part of the piezometric surface. In a section such as figure [2D] the line representing the piezometric surface is the hydraulic gradient of the water in the sand along the section. As there is no flow in the upper sand in this figure, the line representing the piezometric surface is level and extends from the intake area toward the ocean as far as the fresh water extends in the sand.

In the lower sand in figure [2D] the head of the fresh water is sufficient to cause a flow of fresh water into the ocean below sea level, forming a suboceanic fresh-water spring. The fresh water fills the water-bearing formation down to the bottom edge of the overlying impermeable layer and far enough below this level to permit the water to flow out into the ocean. Here again, the salt water fills the lowest part of the formation, but as the pressure in the main body of fresh water is greater than that in the salt water at the outlet, the salt water fills only that part of the formation in which the head of the fresh water has been reduced below the pressure of the salt water by the resistance of the sand to its movement. The line representing the piezometric surface for this sand slopes gently downward from the intake area to the point where the thickness of the sand carrying fresh-water is reduced by the intrusion of salt water. From that point to the point of discharge, the slope increases. . . .

Any general lowering of the head of the fresh water in a sand exposed for a part of its extent to the waters of the ocean will permit the salt water to advance farther inland and occupy more of the sand. The lowering may be caused by natural conditions, such as a dry year or a series of dry years, but lowering due to such causes is not likely to have any serious consequences, unless it occurs in conjunction with artificial withdrawal of water from the sand. This is usually accomplished through wells, either by pumping or by the natural flow from artesian sands. Pumping water from a water-bearing sand lowers the head of the water in it materially in the immediate vicinity of the point of pumping and, to a decreasing extent, for a considerable distance away. If this lowering of head or "cone of depression" occurs above or extends beyond the zone of contact, it will disturb the balance between fresh and salt water and permit the salt water to move up through the formation toward the well. The radius and depth of the cone of influence increase as the rate of pumping from the well is increased . . .

The specific gravity of sea water varies slightly from place to place and sometimes at different depths at the same place, but it is never much greater than that of fresh water. For the purpose of this report, the specific gravity of fresh water may be considered to be 1.000. In the summer of 1913, Bigelow[6] found that the specific gravity of the water off the Atlantic coast of the northern United States at different places and at different depths ranged from 1.019 to 1.028.

Owing to the very small difference between the specific gravity of fresh water and that of salt water, a slight change in the head of the fresh water produces a very considerable change in the position of the zone of contact. If a water-bearing sand is exposed to sea water having a specific gravity of 1.025, the level of the fresh water in it must be maintained at 2.5 feet above mean sea level if the zone of contact is to be held at a depth of 100 feet below sea level. A fresh-water head of 5 feet above mean sea level would be sufficient to hold back the sea water to a depth of 200 feet below sea level. Similarly, if the fresh water head in such a sand were lowered only 2.5 feet, it would permit the salt water to rise 100 feet. If the fresh water head in the sand were lowered to sea level, the salt water would rise to sea level. In a gently sloping confined sand, such as the upper sand in figure [3C], a vertical rise of 100 feet might represent a movement of the salt water several miles inland . . .

It has already been pointed out that salt water fills the sand around and beneath the lens of fresh water under a small island composed entirely of sand. The effect of pumping a well in such a sand island is shown in figure [3A]. As the depth of the zone of contact at any point on this island is determined by the head of the fresh water at that point, any lowering of the water table on the island would cause a rise of the salt water beneath the point where the lowering of head occurred. The section in figure [3A] is shown passing through a shallow well that is being pumped at a rate sufficient to draw the level of the fresh water down to a point somewhat above sea level. The cone of depression caused by pumping this well causes the salt water to rise from beneath the island in a shape similar to the cone of depression but inverted and proportionally much greater in its vertical dimension. This rise in the zone of contact might well be called a salt-water cone of elevation. The distance which the salt water rises is proportional to the amount of lowering of the water table, and the ratio between the lowering of the water table and the rise of the salt water is determined by the relation between the specific gravities of the fresh water and the salt water. The position of the zone of contact beneath the island is not affected outside of the area of the cone of depression produced by pumping the well.

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[6] H. B. Bigelow. Exploration of the coastal water between Nova Scotia and Chesapeake Bay, July and August 1913, by the U.S. Fisheries schooner *Grampus*. Harvard College, Museum Comparative Zoology Bulletin, vol. 69, No. 4, figs. 55, 61. 1915.

A lowering of the water table on the island by the pumping of a well will, of course, produce the same effect whether the well is shallow or deep. As long as the water table is not lowered to sea level, there will be a space filled with fresh water between the bottom of the cone of depression and the peak of the salt water cone of elevation. It is evident, therefore, that a shallow well on such an island might yield fresh water, whereas a deeper well at the same point and pumped at the same rate would penetrate the salt-water cone of elevation and yield salt water . . .

Figure [3B] shows the result of pumping water from a well on a sand island underlain by impervious material. A well very near the coast of such an island would, of course, encounter conditions similar to those in an island composed entirely of sand. Obviously, the safest location for a [fresh-water] well on such an island would be near its center. In this location a well might be pumped at a rate that would draw the water table down even below sea level without drawing in salt water, provided the cone of influence did not extend to any point at which it would be lowering the fresh-water head above the zone of contact. Under such conditions a "fresh water barrier" is said to be maintained between the well and the salt water. If the location of the well or the rate of pumping from it is such that the cone of influence extends beyond the zone of contact, the fresh-water barrier will be broken down, and salt water will be drawn into the well. The diagram shows the conditions that will occur when a well on the island is pumped at a rate which would cause its cone of influence to extend beyond the nearest part of the zone of contact. The salt water would move in under the island and form a cone of elevation beneath the well, similar to the one that would be formed on the sand island shown in figure [3A] except that it would be cut off at the bottom by the impervious layer. Here again a shallow well might yield fresh water, whereas a deeper well at the same place pumped at the same rate would yield salt water . . .

In an artesian sand the pumping of a well may not draw the head down to such a depth that the sand around the well is drained, as under water-table conditions. Usually pumping a well that taps an artesian supply merely lowers the head at and around the well and creates a cone of depression in the piezometric surface without unwatering any of the sand. Figure [3C] illustrates conditions under which salt water might be drawn into such a sand. In the upper sand in the figure a well in the part of the formation that contains salt water would, of course, yield salt water from the beginning. Salt water would be drawn into a well farther inland if the cone of influence of the well extended beyond the edge of the zone of contact. If the well were near the zone of contact, as shown in the diagram, this might occur with only a very slight lowering of the fresh-water head in the formation as a whole.

DEVELOPING THE WELL

From a shallow test well, we found the ground-water level at an average depth of 11 feet, and about 0.9 feet above tide level of the bay. Accordingly, with bay salinities ranging from 20 to 28 parts per thousand, depth of the fresh-water lens was estimated to be from 35 to 45 feet below the ground-water level or from 46 to 56 feet below the surface.

We planned to drill the well to a depth between 50 and 75 feet depending upon the quality of water located and the drilling problems encountered. Because of the fragility of the PVC pipes, the well pipe was water-jetted into place (within the 6-inch iron housing). This process utilizes a bentonite clay and water mixture. Consequently, in sampling the ground water for salinity as drilling proceeded, it was necessary to completely flush the sand of the clay-water mixture by extensive pumping of the well before drawing a sample.

The water, therefore, was tested for salt content at only a few intervals during the jetting operation. Jetting was terminated at 70 feet because of excessive loss of the bentonite mixture through a layer of coarse gravel (more than three-eighths

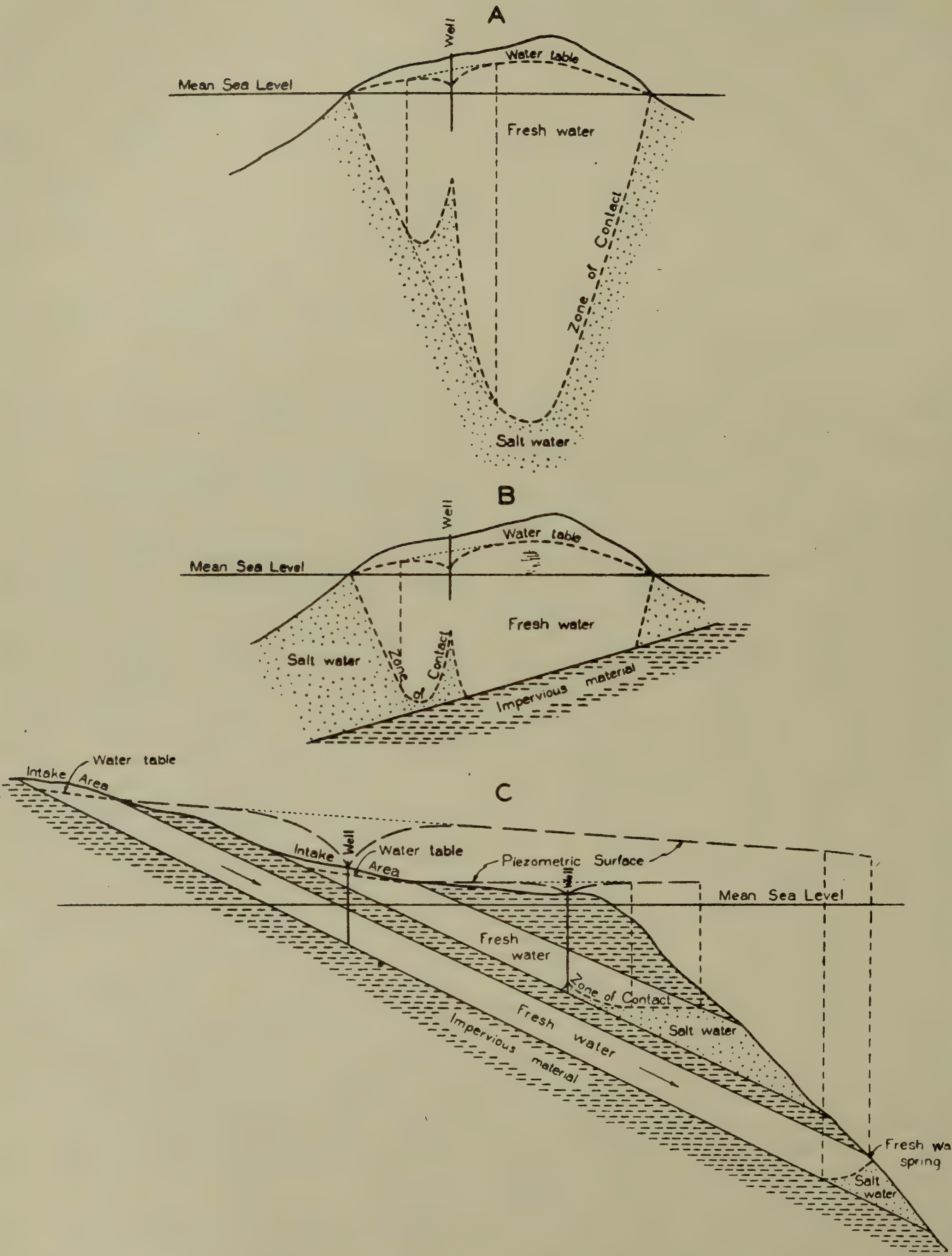

FIGURE 3.—Effect of pumping water from wells in sands exposed to salt water contamination: *A*, in an island composed of sand; *B*, in a sand island underlain by impervious material; *C*, on the coast of a large body of land where the water is confined between impermeable layers. (From Barksdale, Sundstrom & Brunstein, 1936—Supplementary Report on the Ground-Water Supplies of the Atlantic City Region; fig. 6, p. 31.)

inch in diameter) encountered at this depth.

Results of testing confirmed, in general, the theories of Barksdale et al. (1936) regarding depth of the fresh-water lens. This is shown as follows (average ground-water level 11 feet below surface; salinity of the bay 26 to 27 parts per thousand):

At depth of—	*Salinity of well water in parts per thousand*
11 feet	0
27 feet	0
52 feet	20
58 feet	25
65 feet	26
70 feet	23

However, the zone of diffusion was not as narrow as indicated by Barksdale et al. (1936). Another unexpected result was the reduction in salinity below 65 feet, indicating that fresh water was intruding from below, perhaps under artesian pressure resulting from the increase in elevation of the stratum toward the adjoining land mass. The effect of these factors is to confine the zone of higher salinity water to a relatively narrow band. The thickness of this band can be expected to change in response to changes in the salinity of the bay and the height of the fresh-water head above sea level.

On this basis, we decided to establish the well at 60 to 65 feet, but in raising the plastic well pipe from the point of maximum penetration (70 feet) to a shallower depth, the well screen became dislodged. In order to replace this screen, the casing was removed. Unfortunately, it could not be reinstalled to the same depth, and the well screen finally was established at a maximum depth of 57½ feet, where the salinity was only 1 part per thousand less than that of the bay (fig. 4).

After the initial flushing, the water, as it came from the well, was quite clear and appeared at first to meet all quality requirements, but within 4 hours following exposure of the water to air, a brownish-red precipitate appeared rendering the water unsuitable for our use without treatment.

The precipitate proved to be iron at a concentration of 4.8 parts per million, appearing as ferric hydroxide, $Fe(OH)_3$, which resulted from oxidation of soluble ferrous hydroxide, $Fe(OH)_2$.

In the Monmouth County area of New Jersey, iron contamination of potable ground-water supplies of fresh water is a familiar problem. In order to eliminate excessive amounts of iron in domestic water supplies, local communities commonly drill wells to depths of over 700 feet. Fresh-water supplies for Army installations at Sandy Hook are presently taken from wells of 500 to 900 feet in depth to avoid iron contamination, which is very often associated with sands of the Englishtown formation.[7] From logs of wells drilled at Sandy Hook, sands of the Englishtown formation appear to range from 120 to 170 feet below land surface and are underlain by sand and sandy clay to a depth of about 230 feet, which in turn is underlain by clay strata. In places, this clay is a solid, impermeable layer 10-feet thick. This indicates that the source of iron contamination of the sea water supply is from the Englishtown formation above the 230-foot clay layer. Since it is unlikely that iron would occur in the shallow, recent sands (in which the well screen is located), it may be that iron-bearing water is being forced up from the Englishtown formation. The pressure could result from a standing head of water developed over the clay in the adjoining land mass because the strata slope rapidly upward towards the shore.

This is the only explanation of the salinity reversal which appears compatible with the explanations of Barksdale et al. Unfortunately, no funds are available for further explorations. A possible solution to be investigated when funds are available is to obtain water from a shallower point than the present 57½ feet. According to Seymour Subitzky of the U.S. Geological Survey, there is some hope that the amount of ferric iron in solution may decrease rapidly above the point of maximum salinity which is at full bay strength. The upper level of the influence of in-

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[7] The Englishtown formation of late Cretaceous age consists of tan and gray quartz, fine- to medium-grained sand, locally containing beds of clay (Seymour Subitzky, personal communication).

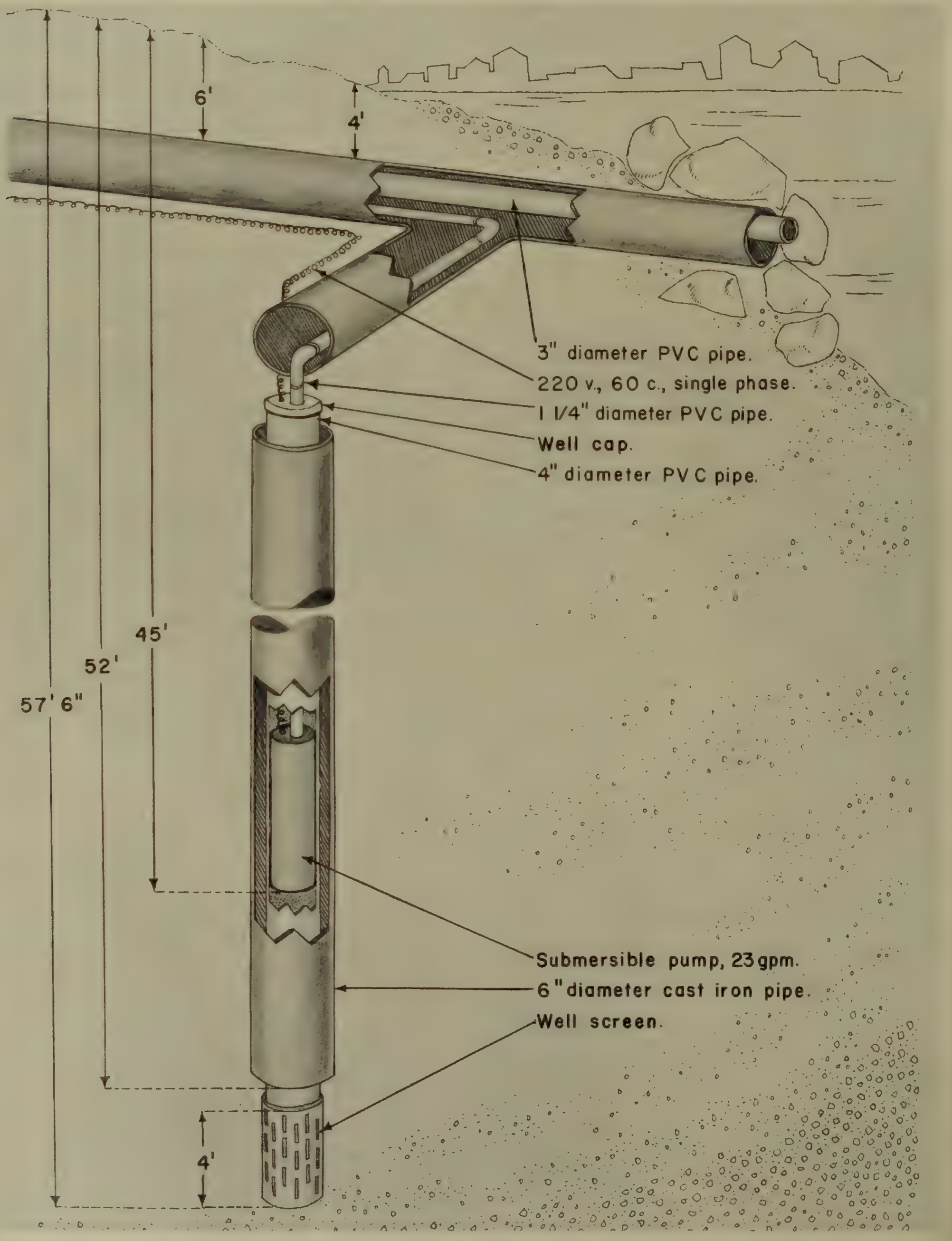

FIGURE 4.—Sea-water well installation at the Sandy Hook Marine Laboratory.

truded iron-bearing water may be associated with the upper level of the coarse-gravel stratum in which the well screen is now located. We hope to relocate the well screen at a higher point where we may find sea water of acceptable salinity, but with a ferrous iron content below the precipitation threshold—about 3 parts per million.

Although we have not tested the production capacity of the well, it can be assumed that the present installation, with a larger pump and transmission line, could produce nearly 100 gallons a minute without excessive drawdown.

CHARACTERISTICS OF THE WELL WATER

In order to use the well water, it is necessary to allow the ferric hydroxide to settle. This has been accomplished by holding batches of water in a 2,000-gallon tank. Since the flocculate is very light, requiring a week or more from time of oxidation to settling, this is a slow process. After settling, however, the water has been used successfully to rear eggs, larvae, juveniles, or adults of 51 species of 31 families of fishes. No failure to maintain any species of fish can be attributed to the quality of the decanted water. Many groups of invertebrates (ctenophores, coelenterates, mollusks, echinoderms, crustaceans) and several species of algae also have been successfully maintained in the well water. The following analysis of the well water (March, 1962) shows that after precipitation no iron remains in solution:

	Parts per million
Fe in solution, after settling	0.00
Mn in solution, after settling	.19
Fe, before settling	4.8
Mn, before settling	.29
SiO_2, before settling	8.8
Chloride, before settling	10

The temperature of the well water, taken during the occasional pumping intervals between September 1961 and March 1962, has remained at 56.3°±0.2° F. Unfortunately, owing to the settling procedure, the constant temperature factor is lost and room air-conditioning is used as a substitute. When the water is ready for use after settling, pH ranges from 7.6 to 8.1, dissolved oxygen is about 6 p.p.m., and specific conductance 28,500 micromhos (at 25° C.).

DISCUSSION

In view of its many advantages, it is suggested that the use of a ground-water source of sea water be explored when planning a supply system for any seaside laboratory. The feasibility of a ground-water supply will depend upon local conditions, and few generalizations can be drawn. Generally, if underlying earth material is uniformly permeable and extends laterally to the surrounding sea water, a sufficient supply can be obtained by penetrating to a depth beneath the fresh-water layer overlying the sea water. There remains, as a major problem, the possibility of chemical contamination of the ground-water source.

Except for the iron problem, Sandy Hook is an ideal location for a shallow salt-water well. In many other areas also, ground sources are used to supply aquariums. One example is the well, described in two papers in this collection (Strasburg, Nakamura) which was drilled into a coral formation in Honolulu. There is also a well in use at the Waikiki Aquarium in Honolulu. Spencer Tinker, Director of the Aquarium, has given us the following information about this well:

> The Aquarium stands on the edge of the ocean just a few feet from the water. The land under the Aquarium is loose unconsolidated rubble, sand, etc., to a depth of 40 feet. From 40 feet downward for an unknown distance there is an ancient coral reef which is quite solid but which contains scattered sand pockets and which is permeated by sea water. The Aquarium bored a hole in this area 80 feet in depth. This hole is larger than a foot in diameter and has the upper 40 feet cased with a 12-inch wrought-iron pipe. This pipe or casing keeps the rubble from

caving into the well, but there is no casing in the lower half of the well where the hole enters into the ancient coral reef. The water from the buried coral reef enters the lower open part of the well and rises up the casing and stands at tide level. The Aquarium pumps this water out of the casing and it continually replaces itself from the coral reef beneath. This 12-inch well cost $1,600 and took about 4 or 5 days to drill. We pump 500 to 600 gallons a minute from it, but it will yield in excess of 1,000 gallons. When the pumping rate approaches 1,000 gallons a minute, the drawdown approaches 2 feet. The quality of this water is good. It is absolutely clear, without larvae of any kind, free from seaweed, and makes us independent of storms and tides. It is more saline than the water on the shoreline and compares in salinity with the water far at sea. It is about 1° or so cooler than the shoreline water. Its great disadvantage, if this be one, is that it contains no oxygen. The oxygen in this water measures about 0.1 cc. per liter. This, of course, is insufficient for any biological work. We do imagine, however, that this lack of oxygen may prolong the life of various things that rust. To restore the oxygen into this water, we let the water draw in air as it comes forth from the valve above the fish tank. This simple act lifts the oxygen content to about 2.5 cc. per liter. This is not enough, perhaps, but it gets better with use and aeration.

These wells can be drilled close together. Eight or ten feet apart is sufficient distance to provide a well of the same capacity as the first one. These can be drilled into lava rock as well as into the coral reef.

The New York Aquarium on the beach at Coney Island also uses a ground-water supply. All attempts to pump water directly from the sea have been abandoned in favor of two 12-inch-diameter wells drilled to a depth of just over 200 feet, into a stratum of coarse gravel which provides excellent sea water. Each well is capable of producing 600 gallons a minute. The major problem associated with the Coney Island wells is that the water must be oxygenated. The New York Aquarium, which is about 7½ nautical miles north of the Sandy Hook Marine Laboratory, has no iron problem with its wells.

Efforts to locate a subsurface source of sea water at the Miami Marine Laboratory were unsuccessful (see Wisby, this volume) because of contamination of the ground water by H_2S.

At the time of writing, the Narragansett Marine Laboratory at Kingston, R.I., was in the process of developing a ground-water supply of sea water. The plans for this system called for a series of collecting wells from which the water was to flow by gravity through pipes to a central pump well. The water was to be pumped from the central well to a storage tank from which the aquariums were to be supplied by gravity.

EQUIPMENT SUPPLIERS

The major suppliers of components of our sea water system are listed below:

Submersible pump—Model 75 K1–5Ca (This model contains some metallic parts and is in use temporarily until the all-plastic model, 75 M1–5Cb, becomes available): Red Jacket Mfg. Co., Davenport, Iowa.

Water-supply pipe and fittings—Kraloy Type II, Schedule 80, pipe and fittings (3-inch-diameter, thick-wall, threaded PVC pipe and 1¼-inch-diameter PVC pipe): Kraloy/Chemtrol Co., Santa Ana, Calif.

Drain line and fittings—"Tuftite" PVC Plastic Sewer and drain pipe (During the course of our well-drilling operation we changed from the aforementioned Kraloy/Chemtrol Company to this manufacturer for the following reasons: (*a*) A branch office located nearby, therefore involving lower shipping expenses, and (*b*) a wider selection of material, particularly fittings. Lower prices. All pipe and fittings could have been obtained from this source.)

Well screen: Gator Sales Co., Shreveport, La.

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PRACTICAL SUGGESTIONS FOR CONSTRUCTION AND MAINTENANCE OF LARGE-VOLUME SEA-WATER SYSTEMS

By John T. Hughes, ***Marine Biologist***

Massachusetts Division of Marine Fisheries, Vineyard Haven, Mass.

Abstract.—A brief description is given of a sea-water supply system in use in a lobster hatchery and research station in a region where the winters are severe. Two electrically driven pumps supply a maximum of 900 gallons per minute to an overhead tank holding 15,000 gallons. The water flows by gravity to station aquariums. Thirty-four concise suggestions are presented to allay problems of fouling, siltation, equipment breakdown, and toxicity.

The Commonwealth of Massachusetts built a sea-water system in 1948 to supply water to the new lobster hatchery and research station located in Oak Bluffs on the island of Martha's Vineyard. The salt-water system supplying the station consists of two 450-gallons-per-minute cast-iron centrifugal pumps, each with its own 6-inch plastic suction line and cross-valved so that both pumps can use either suction line. The suction lines are approximately 400 feet long lying on the bottom of the sea, with the foot valve supported 5 feet off the bottom in 10 feet of water. The foot valves are kept off the bottom by lashing them to pilings, the tops of which are below the surface of the water at the lowest tide level in order to prevent the ice from moving them in the winter.

Just before the suction lines enter the pumps, each line has a basket strainer to catch mussels or other fouling organisms which might plug the pump impeller. The pumps alternate in filling the storage tank when the water level drops 3 feet. The pumps are electrically driven; one pump is made so that it can be coupled to an auxiliary gasoline engine. The water is pumped into a cylindrical 15,000-gallon steel tank which is supported 15 feet off the ground. The water then passes to the station by gravity through a 6-inch pipe from which 2-inch lines lead to the various tank systems.

The following is a list of methods and features which are suggested, on the basis of our experience, to combat problems, such as fouling, siltation, equipment breakdown, and toxicity of components.

1. After one has carefully figured out what size pipe and pump will be necessary to supply sea water to an installation, double the size of everything.
2. Install one more pump than is necessary to take care of all possible emergencies.
3. Have a separate suction line for each pump and have each suction line cross-valved (with good high-pressure valves) so that any suction line may be used with any pump.
4. Use plastic pipe for suction and discharge lines. Use no pipe with copper.

Contribution No. 10 of the Division of Marine Fisheries, Massachusetts Department of Natural Resources, Boston, Mass.

5. Use flanged fittings with brass or stainless-steel bolts and neoprene washers.

6. Use tees for elbows, crosses for tees, and have flanged unions in every line.

7. Make positive that there are no air leaks on the suction side of the pumps.

8. Any pilings used to support pipe in the water should have their tops a few inches below low-water mark to prevent the ice from removing them.

9. Mussels can be killed by closing off a suction line for a few weeks and using another line. (No steam can be used with the plastic pipe of today.) No chemicals should be used to kill fouling animals.

10. Use no lead- or copper-base paints in tanks. Use only plastic-base paints.

11. Pump to a storage tank, and feed station by gravity.

12. Have the line leading from the storage tank to station 2 feet above the floor of the storage tank.

13. Use fiberglass tanks and holding units.

14. Do not use lindane or similar insect sprays.

15. Install an emergency generating plant to take care of lights and a minimum of one pump.

16. Have one pump capable of being coupled to a gasoline engine.

17. Have an emergency bypass from one pump to station without going through the storage tank.

18. Have as short a suction line as possible. Pump from a sump which is fed from deep water through a 5-foot pipe.

19. Have an automatic alarm system to warn of low water in storage tank. Telephone lines can be rented to ring an alarm in several isolated homes or offices.

20. Have gages on suction and discharge sides of pumps to indicate pump failures and breakdowns.

21. To clean growth from insides of some pipes, tie old tire chains on a rope and pull through. Have a rope on both ends so that the chain can be pulled back if it gets stuck.

22. Use as few values and fittings on suction side of pump as possible, and those used should be of high grade.

23. Have a basket strainer on suction line just before pump to catch detritus and mussels that would otherwise get plugged in pump impellers.

24. Use circuit breakers instead of fuses.

25. Drill $\frac{1}{8}$ inch holes in the flapper of the check valve on the discharge side of the pump so that enough water can pass back through the pump rather than through the packings.

26. Put as few pipes as possible under the ground.

27. Make the frost-proofing boxes easy to take apart.

28. When passing through a bulkhead, overhead, or floor with a pipe, leave plenty of room to disassemble, particularly when through cement.

29. Make sure electrical system has a good ground, and protect from lightning.

30. When installing drainage system, use a few crosses with blank faces for future additions.

31. Have plenty of pitch to the floor toward drains.

32. Do not cover with cement the pipes that are to be used for heating or cooling water.

33. Use the type of pipe supports that can be easily taken apart and reused.

34. Where Y-type sediment strainers are to be used, have them made of plastic (PVC) pipe and perforated sheeting. Sediment strainers should be three times the size of the pipe on which they will be used.

NARRAGANSETT MARINE LABORATORY SEA-WATER SYSTEM

By Charles J. Fish, *Director*
Narragansett Marine Laboratory, University of Rhode Island, Kingston, R.I.

Abstract.—The stormproof sea-water supply and distribution system of the University of Rhode Island's Narragansett Marine Laboratory comprises six intake wells along the shoreline, a pump well, a pipeline to an elevated receiving tank, and a distributing pipe system from tank to laboratories. The system delivers about 120 gallons a minute during pumping periods.

Installation of a salt-water supply and distribution system on the Narragansett Bay Campus of the University of Rhode Island has been complicated by (1) its easterly exposure which in the past necessitated frequent storm damage repairs, and (2) seaward runoff of fresh ground water in the upper bottom layer which precluded use of well-points for intake filtration. Filtration has been found necessary in the bay to prevent fouling growth in the pipes.

To overcome these obstacles the present stormproof system has been devised during the past year and is now in successful operation. Grateful acknowledgment is made for the valuable services of Mr. Clarence Ebaugh, Consulting Engineer, whose examination of the beach fronting the property established that an adequate supply of Bay water would percolate freely through the sand and gravel bottom sediment to his recommended porous intake pipe and well, and Mr. Walter Caccia, University Hydraulic Engineer, who modified the original shoreline installation plan, designed the balance of the system, and supervised its installation.

As shown in figure 1, the system consists of four major divisions, a unit of six intake wells, a pump well, a Transite pipeline leading from the pump well to an elevated receiving tank, and a distributing pipe system from the tank to the laboratories.

Each of the six intake wells (fig. 2) consists of a cylindrical Transite pipe within a larger pipe of the same material, with the annular space between the two filled with graded crushed stone to act as a filter. The external piping is 36 inches in inside diameter and perforated with 1-inch holes on 3-inch centers. The inside piping is 10 inches inside diameter with ¼-inch perforations on 2-inch centers. This inner pipe collects and conducts the water from the crushed-stone filter to the manifold leading to the pump chamber.

In installing the intake wells the following steps were taken: (1) Building of a seaward sand and gravel barrier dike sufficiently offshore to permit dredging a crater to a depth of 12 feet, (2) arranging the work schedule to coincide with the time of the falling tide and period of low water, (3) providing sufficient pumping capacity to drain the crater as fast or faster than the fall of the tide, for maintenance of a water-free excavation, and (4)

Contribution No. 7 from the Narragansett Marine Laboratory of the University of Rhode Island.

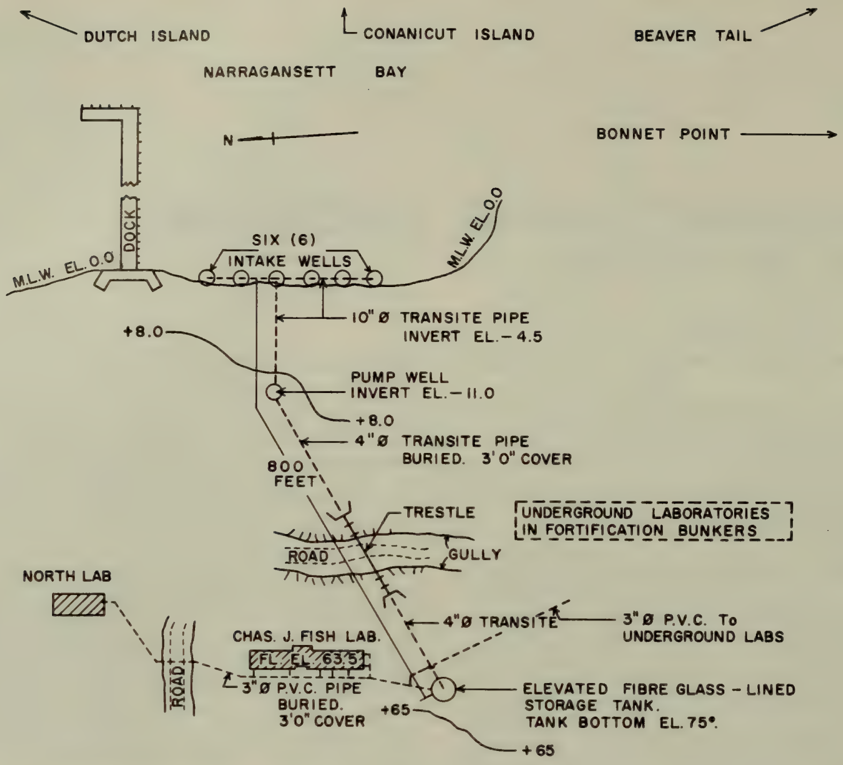
DUTCH ISLAND
CONANICUT ISLAND
BEAVER TAIL
NARRAGANSETT BAY
N
BONNET POINT
M.L.W. EL. 0.0
DOCK
SIX (6) INTAKE WELLS
M.L.W. EL. 0.0
10" Ø TRANSITE PIPE INVERT EL. – 4.5
+8.0
PUMP WELL INVERT EL. – 11.0
+8.0
4" Ø TRANSITE PIPE BURIED. 3' 0" COVER
800 FEET
TRESTLE
UNDERGROUND LABORATORIES IN FORTIFICATION BUNKERS
ROAD
GULLY
NORTH LAB
4"Ø TRANSITE
3" Ø P.V.C. To UNDERGROUND LABS
CHAS. J. FISH LAB.
FL. EL. 63.5
ROAD
3" Ø P.V.C. PIPE BURIED. 3'0" COVER
+65
ELEVATED FIBRE GLASS – LINED STORAGE TANK. TANK BOTTOM EL. 75'.
+65

Figure 1.—Salt-water system at University of Rhode Island Graduate School of Oceanography, Saunderstown, R.I.

assembling of the entire intake well unit on shore and placement in the drained crater with a crane.

The pump well (fig. 2) accommodates one Flygt Bibo 4 submersible pump. A second pump is held in reserve for periodic rotation or quick replacement in case of emergency. This is facilitated by having each pump fully equipped with its discharge piping. Ever-tite aluminum rapid couplings and detachable electrical cables connect the pumps and receptacles, thus simplifying the task of making a pump change. The pump is of compact design weighing 170 pounds. It is 29 inches in height and 19 inches in external diameter.

The pipeline conveying water from the pump well to the elevated receiving tank is entirely self-draining without valleys or dips that would retain water between pumping periods. A check valve and a vacuum breaker in the line, just before entering the receiving tank, permit the line to empty itself entirely through the pump chamber within a few minutes after cessation of pumping. This self-draining feature of the line eliminates the necessity of insulating it against freezing. The line, 750 feet in length, is of 4-inch Transite pipe equipped with rubber ring couplings. Valves and checks are equipped with Buna-N seats and seals.

The receiving tank (fig. 3) is constructed of wood with a lining of 4-ply fiberglass. The supply and discharge pipes pass vertically through the bottom and are also of fiberglass. Two mercury float switches in the tank control starting and stopping of the water inflow, operation of the pump being electrically automatic.

The distribution pipes from the receiv-

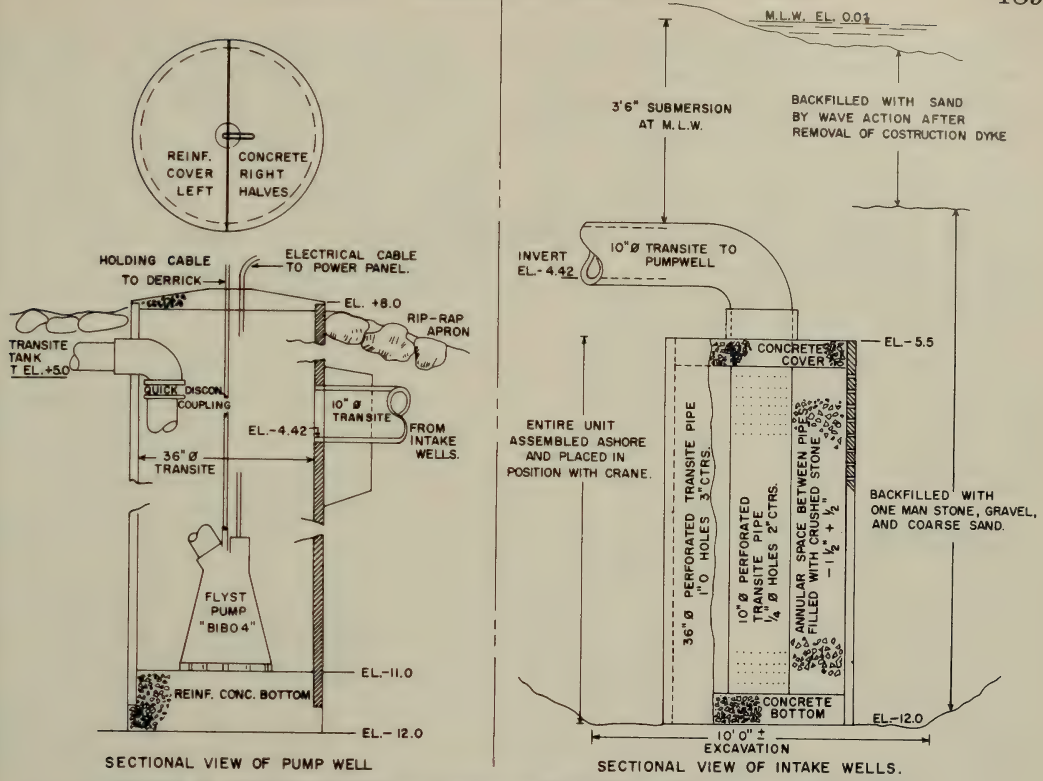
REINF. CONCRETE COVER LEFT RIGHT HALVES
HOLDING CABLE TO DERRICK
ELECTRICAL CABLE TO POWER PANEL.
EL. +8.0
RIP-RAP APRON
TRANSITE TANK T EL.+5.0
QUICK DISCON. COUPLING
10" Ø TRANSITE
FROM INTAKE WELLS.
EL.-4.42
36" Ø TRANSITE
FLYST PUMP "BIBO4"
EL.-11.0
REINF. CONC. BOTTOM
EL.- 12.0
SECTIONAL VIEW OF PUMP WELL
M.L.W. EL. 0.0±
3'6" SUBMERSION AT M.L.W.
BACKFILLED WITH SAND BY WAVE ACTION AFTER REMOVAL OF COSTRUCTION DYKE
INVERT EL.- 4.42
10"Ø TRANSITE TO PUMPWELL
CONCRETE COVER
EL.- 5.5
ENTIRE UNIT ASSEMBLED ASHORE AND PLACED IN POSITION WITH CRANE.
36" Ø PERFORATED TRANSITE PIPE 1"Ø HOLES 3" CTRS.
10"Ø PERFORATED TRANSITE PIPE 1/4" Ø HOLES 2" CTRS.
ANNULAR SPACE BETWEEN PIPES FILLED WITH CRUSHED STONE - 1 1/2" + 1/2"
BACKFILLED WITH ONE MAN STONE, GRAVEL, AND COARSE SAND.
CONCRETE BOTTOM
EL.-12.0
10' 0" ± EXCAVATION
SECTIONAL VIEW OF INTAKE WELLS.

FIGURE 2.—Sectional views of pump well (left) and intake wells (right).

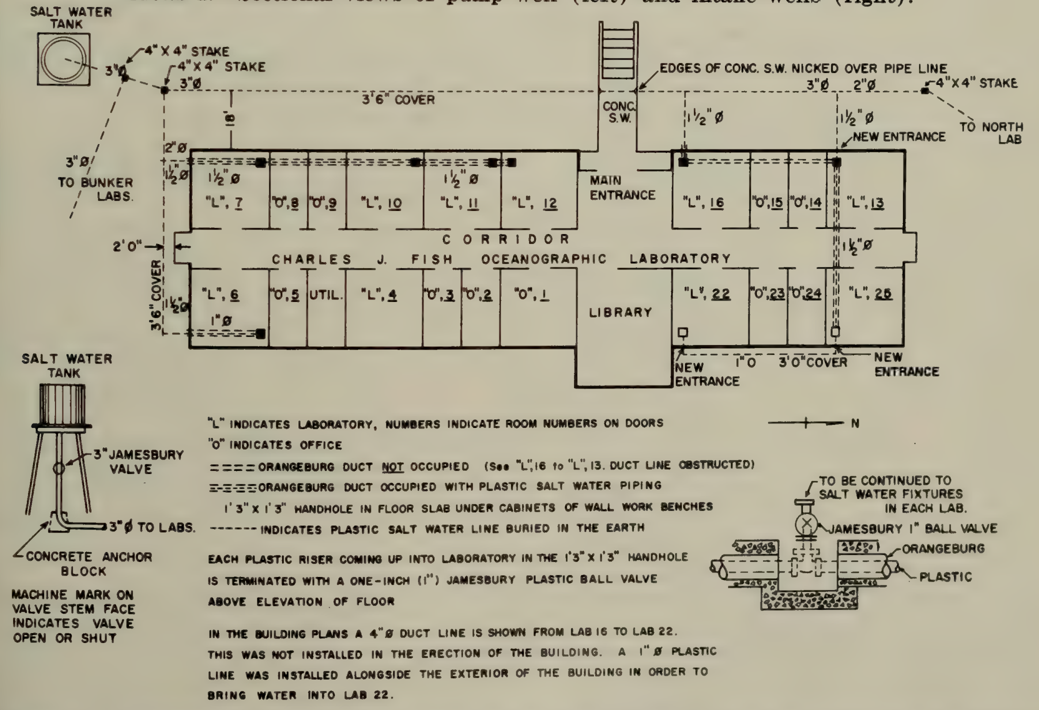
SALT WATER TANK
4" X 4" STAKE
3"Ø
4" X 4" STAKE
3"Ø
3'6" COVER
18"
EDGES OF CONC. S.W. NICKED OVER PIPE LINE
3"Ø 2"Ø
4"X4" STAKE
TO NORTH LAB
CONC. S.W.
1 1/2" Ø
1 1/2" Ø
NEW ENTRANCE
3"Ø TO BUNKER LABS.
2"Ø
1 1/2"Ø
1 1/2" Ø
1 1/2" Ø
"L", 7
"O", 8
"O", 9
"L", 10
"L", 11
"L", 12
MAIN ENTRANCE
"L", 16
"O", 15
"O", 14
"L", 13
CORRIDOR
CHARLES J. FISH OCEANOGRAPHIC LABORATORY
2'0"
1 1/2"Ø
3'6" COVER
"L", 6
"O", 5
UTIL.
"L", 4
"O", 3
"O", 2
"O", 1
LIBRARY
"L", 22
"O", 23
"O", 24
"L", 25
1 1/2"Ø
1"Ø
NEW ENTRANCE
1"Ø
3'0" COVER
NEW ENTRANCE
SALT WATER TANK
3" JAMESBURY VALVE
3"Ø TO LABS.
CONCRETE ANCHOR BLOCK
MACHINE MARK ON VALVE STEM FACE INDICATES VALVE OPEN OR SHUT
"L" INDICATES LABORATORY, NUMBERS INDICATE ROOM NUMBERS ON DOORS
"O" INDICATES OFFICE
N
==== ORANGEBURG DUCT NOT OCCUPIED (See "L", 16 to "L", 13. DUCT LINE OBSTRUCTED)
=-=-=- ORANGEBURG DUCT OCCUPIED WITH PLASTIC SALT WATER PIPING
1'3" X 1'3" HANDHOLE IN FLOOR SLAB UNDER CABINETS OF WALL WORK BENCHES
------ INDICATES PLASTIC SALT WATER LINE BURIED IN THE EARTH
EACH PLASTIC RISER COMING UP INTO LABORATORY IN THE 1'3" X 1'3" HANDHOLE IS TERMINATED WITH A ONE-INCH (1") JAMESBURY PLASTIC BALL VALVE ABOVE ELEVATION OF FLOOR
IN THE BUILDING PLANS A 4"Ø DUCT LINE IS SHOWN FROM LAB 16 TO LAB 22. THIS WAS NOT INSTALLED IN THE ERECTION OF THE BUILDING. A 1" Ø PLASTIC LINE WAS INSTALLED ALONGSIDE THE EXTERIOR OF THE BUILDING IN ORDER TO BRING WATER INTO LAB 22.
TO BE CONTINUED TO SALT WATER FIXTURES IN EACH LAB.
JAMESBURY 1" BALL VALVE
ORANGEBURG
PLASTIC

FIGURE 3.—Diagrammatic sketch showing salt water distribution in Charles J. Fish Oceanographic Laboratory (not to scale).

ing tank to the working stations in the laboratories are of polyvinyl chloride. Each station is provided with Jamesbury plastic ball valves (fig. 3) and terminates with plastic faucets and swivel goosenecks. The plastic faucets are provided with plastic side valves for connection of flexible lines to laboratory aquaria.

Pumping against a head of 75 feet and through the 4-inch Transite line approximately 750 feet in length, the system is now delivering approximately 120 gallons per minute during the pumping periods. Added intake wells could be installed if an increased water supply should be required at some future time.

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SOME CHRONIC EFFECTS OF DDT ON CUTTHROAT TROUT

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RESEARCH REPORT 64

RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two Bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

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SOME CHRONIC EFFECTS OF DDT ON CUTTHROAT TROUT

Don Allison, Burton J. Kallman
Oliver B. Cope, and Charles Van Valin

RESEARCH REPORT 64

UNITED STATES DEPARTMENT OF THE INTERIOR
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Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*
FISH AND WILDLIFE SERVICE
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Bureau of Sport Fisheries and Wildlife
Daniel H. Janzen, *Director*

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ABSTRACT

Effects on cutthroat trout (*Salmo clarki lewisi*) of periodic exposure to different levels of DDT in bath and in food were determined over a 20-month period involving one spawning cycle. High dosages produced a marked increase in mortality, probably by reducing resistance to nonspecific stressors; surviving fish in high-dosage lots were significantly larger than those in control and low-DDT lots. This effect occurred with no change in growth rate, perhaps because of removal of smaller and less vigorous fish by DDT. The number and volume of eggs produced was not reduced by DDT, but mortality among sac-fry appeared to be highest in the high-dosage lots. The reproductive potential of fish treated at high levels was reduced by high rates of mortality among adults and in sac-fry from survivors. Intermediate and low dosages of DDT generally did not measurably affect mortality, growth, or reproductive potential, indicating that a threshold level exists around 0.1 p.p.m. monthly in contact form and around 0.3 mg. DDT per kg. of fish weekly in the diet for the toxic effects of DDT. Metabolic conversion of DDT to DDE occurred to a greater relative extent in fish exposed to low dosages; at these levels, total residues of chlorinated hydrocarbon were not higher than in controls. Residues of DDD were present in most of the fish, possibly as a metabolite of DDT. These data suggest that the usual control treatment exposures to sublethal levels of DDT are unlikely to damage a population of rainbow trout.

SOME CHRONIC EFFECTS OF DDT ON CUTTHROAT TROUT

By Donald T. Allison, *Fishery Research Biologist,* Burton J. Kallman, *Chemist,* Oliver B. Cope, *Fishery Research Biologist,* and Charles Van Valin, *Chemist*

Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife

DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, is the most widely used chemical insecticide in the United States, partly because of its low cost, partly because of its insecticidal efficiency, and because of its relatively low acute toxicity to vertebrate animals. The literature on the insecticidal properties of this chemical is voluminous, and some data are available on its acute toxicity for mammals (Neal et al. 1946; Phillips and Gilman, 1946), birds (Coburn and Treichler, 1946), and fish (Henderson, Pickering, and Tarzwell, 1959).

The oral acute mean lethal doses reported for mammals range from 150 milligrams per kilogram of body weight in rats (Smith and Stohlman, 1944) to 500 mg./kg. in man (Negherbon, 1959). For birds the reported acute mean lethal doses extend from 60 mg./kg. in oil in the diet of quail (Coburn and Treichler, 1946) to 600 mg./kg. in gum acacia in the diet of young pheasants (DeWitt, Derby, and Mangan, 1955); the TLm (the concentration which half the population can tolerate) values listed for fish range from 0.01 part per million (in the medium) for rainbow trout to 0.1 p.p.m. for goldfish (Odum and Sumerford, 1946).

Large doses in mammals yield the neurologic syndrome of initial hyperexcitability, nervousness, eyelid twitching, progressing to severe and general twitching, giving way to tonic-clonic convulsions, followed by more prolonged twitchings and convulsions, and attended by difficulties of breathing. Abrupt culmination of the convulsive phase leaves the animal exhausted and motionless. Repeated doses may resolve to final, continuous, and severe tremor, with coma preceding death. In subacute dosage, effects such as hemoglobin decline with hypochromic anemia reflect a dietary cause due to prolonged appetite loss and inadequate food intake in the periods of tremor and convulsion. Some of the acute responses observed in fish are erratic swimming, loss of equilibrium, and hyperventilation.

The acute toxicity of DDT to vertebrates is relatively low, and acute mortality of vertebrates in the United States from exposure to DDT is probably not great, considering the large quantities of this chemical that are used each year. However, there is some concern over the possibilities that subclinical damage will occur as a result of exposure to low concentrations and not be recognized.

The Fish-Pesticide Research Laboratory of the Bureau of Sport Fisheries and Wild-

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NOTE.—Present addresses of the authors are as follows: Donald T. Allison, Fish-Pesticide Research Laboratory, Jackson, Wyo.; Dr. Burton J. Kallman, Veterans Administration Center, Wadsworth Hospital, Wilshire and Sawtelle Blvds., Los Angeles, Calif.; Dr. Oliver B. Cope and Charles Van Valin, Fish-Pesticide Research Laboratory, Bldg. 45, Federal Center, Denver, Colo.

life organized a series of experiments at the National Fish Hatchery at Jackson, Wyo., in 1960 to measure some chronic effects of the exposure of cutthroat trout, *Salmo clarki lewisi*, to DDT. Comparisons between untreated fish and fish exposed at different levels of DDT were made over a 20-month period.

The authors are indebted to several persons who assisted on the project in various ways. Joseph Mazuranich made facilities available at the National Fish Hatchery at Jackson, Wyo. John O'Donnell, Thomas Shen, Robert Kroesen, Diane Dwyer, and Robert Slater worked on residue analyses. W. R. Bridges, Austin Andrews, Joseph McCraren, and Vernon Bressler assisted in field operations and on logistic problems. Dr. Louis Locke made histopathology examinations of tissues.

BACKGROUND

The experiments began on December 8, 1960, and were designed to measure effects on cutthroat trout of DDT [1] taken through ingestion and through contact in water. Eleven lots of yearling Snake River cutthroat cultured in the National Fish Hatchery at Jackson, Wyo., were used, each lot containing 636 fish. One lot was an untreated control; five lots were exposed every 28 days to a 30-minute bath of DDT in 55-gallon drums coated with nontoxic asphalt paint, each lot at a given sublethal concentration; five lots were given DDT once a week in a pelleted diet, each lot receiving a given sublethal concentration; the fish averaged 6.5 inches in length, and were 21 months old when the experiment began. They were randomly divided into the 11 lots, each lot was divided into 3 sublots with equal numbers of fish, and the sublots were randomly distributed in 33 pens in 2 raceways. All 33 sublots received the same cultural treatment through the experiment, except that the fish given DDT baths received extra handling during the baths and some fish received extra handling during artificial spawning. Sample fish were withdrawn according to a schedule and were either frozen for chemical analysis for DDT residues or fixed in Bouin's solution for histopathological examination. Sublots were weighed periodically, and sample fish and those that died during the experiment were individually weighed, sexed, measured, and examined for gross pathology. Microhematocrit measurements were made from sample fish. Fish were spawned in early 1962 and the eggs were cultured in jars. The progeny from the various sublots and from different spawnings were developed and their identities retained.

The fish were fed a commercial dry diet with meat supplement before initiation of the experiment.[2] The basic diet during the experiment was a dry pelleted formulation having the following composition: Whitefish meal, 24 percent; wheat middlings, 24 percent; distiller's solubles, 21 percent; cottonseed meal, 15 percent; brewer's yeast, 10 percent; skim milk, 3 percent; cod-liver oil, 3 percent. To each 100 pounds of the mixture was added 2 pounds of corn sirup and 6 pounds of water and dye. The diet was supplemented with beef liver and spleen 2 days a week. The fish receiving DDT in the diet had the following amounts incorporated in the pellets every 7 days, one level for each lot: 3.0 milligrams per kilogram of body weight; 1.0 mg./kg.; 0.3 mg./kg.; 0.1 mg./kg.; and 0.03 mg./kg. (table 1).

The fish exposed to DDT by contact in baths were exposed to the following amounts, added in acetone solution, one level for each lot of fish: 1.0 part per million; 0.3 p.p.m.; 0.1 p.p.m.; 0.03 p.p.m.; and 0.01 p.p.m.

---

[1] p,p'-DDT, 100-percent reference standard, Nutritional Biochemicals Corp., was used.

[2] Comment on chlorinated hydrocarbon residues in the commercial diets is given on p. 15.

Sample fish were taken in the following schedule: Immediately before the experiment began; 3, 7, 14, 27, 56, and 111 days after the experiment began; and about every 56 days thereafter (table 2). The experiment terminated after 16 samples had been taken, 612 days after the experiment began. From each sublot sampled, two fish were taken for chemical analysis, and two for pathology work.

TABLE 1.—*DDT treatments given experimental lots*

Lot	DDT treatment
Control:	
Lot I	None
DDT in water bath:	*Parts per million*
Lot II	1.0
Lot III	.3
Lot IV	.1
Lot V	.03
Lot VI	.01
DDT in feed:	*Milligrams per kilogram of body weight*
Lot VII	3.0
Lot VIII	1.0
Lot IX	.3
Lot X	.1
Lot XI	.03

TABLE 2.—*Sampling schedule*

Sample	Date	Days elapsed	Cumulative number of feedings of DDT	Cumulative number of exposures to DDT in bath
Sample 1	Dec. 6, 1960	--------	0	0
Sample 2	Dec. 11, 1960	3	1	1
Sample 3	Dec. 15, 1960	7	2	1
Sample 4	Dec. 22, 1960	14	3	1
Sample 5	Jan. 4, 1961	27	4	1
Sample 6	Feb. 2, 1961	56	9	2
Sample 7	Mar. 29, 1961	111	16	4
Sample 8	May 24, 1961	166	19	6
Sample 9	July 18, 1961	221	27	8
Sample 10	Sept. 13, 1961	278	34	10
Sample 11	Nov. 8, 1961	334	42	12
Sample 12	Jan. 4, 1962	391	49	14
Sample 13	Feb. 28, 1962	446	56	14
Sample 14	Apr. 20, 1962	497	63	14
Sample 15	June 21, 1962	559	72	16
Sample 16	Aug. 13, 1962	612	79	18

MORTALITY

During the course of the experiment, the fish were treated with nf-180 for furunculosis, sulfamerazine for furunculosis and kidney disease, formaldehyde for fungus and animal parasites, acetic acid for *Epistylis,* and malachite green for fungus after spawning. A recent analysis of the water at Jackson indicated a pH of 7.8, total dissolved solids of 209 p.p.m., hardness as $CaCO_3$ of 168 p.p.m., and a specific conductance of 340 micromhos at 25° C.

All of the fish which died during the course of the experiment were retained. Relatively small numbers were collected in a moribund condition and included for analysis.

It was general practice to record the date of death for each fish as well as its weight, standard length, sex, sexual development, and somatic condition. In this manner records were kept for approximately 3,000 dead fish during the 20-month experimental period. In a few cases some of the recorded data were incomplete or questionable and could not be used in certain phases of the analysis. This accounts for the discrepancy between the numbers used for cumulative mortality and those used for sex ratios.

The 33 sublots were located randomly in the two raceways. Among the three sublots of any given lot, mortality was usually a little higher in the sublot located at the lower end of a raceway. However, because the sublots were generally well scattered, this bias applied throughout all treatment levels. To simplify analysis, data from sublots have been combined and are presented as totals for each of the treatment lots.

The mean values used for cumulative mortality were subjected to *t* tests at 2-month intervals to determine whether the values for treated lots were significantly different from those of the control.

RESULTS

Cumulative Mortality

Cumulative mortality for lots exposed to DDT in bath and in food is presented graphically in figure 1. Although the mortality differs among lots, the mortality curves are very similar in general configuration. Fluctuations in mortality are reflected throughout most of the lots on about the same dates.

DDT in bath.—Absolute mortalities for lots II (1.0 p.p.m. DDT) and III (0.3 p.p.m. DDT) were almost identical, with only a slight temporal and numerical lag on the part of lot III. The cumulative mortality in lots II and III differed significantly from that of the control after the fourth month of exposure. By 6 months, mortality in lot II was significantly different from that of the control ($P<0.005$), and the same applied to lot III ($P<0.001$). About three times as many fish died in lots II and III as in the control group after 1 year of treatment until the end of the experiment.

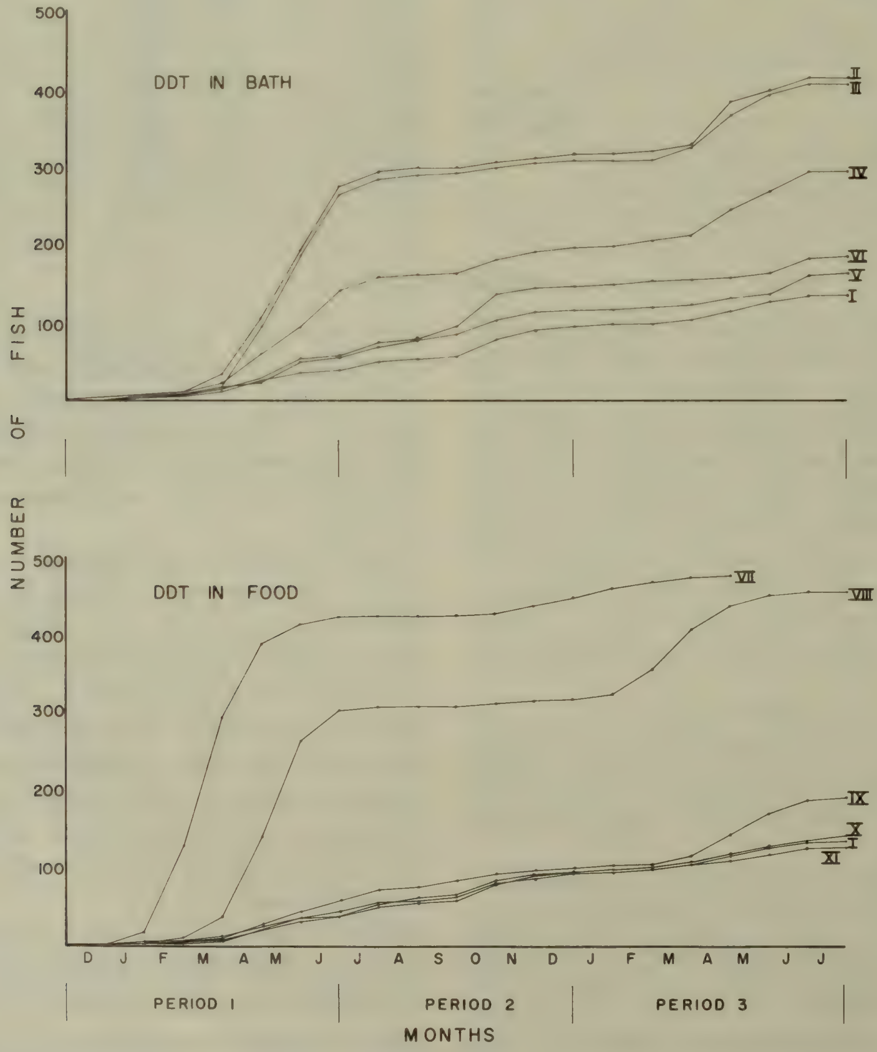


FIGURE 1.—Cumulative mortality. Each lot contained 636 fish.

Mortality in lot IV remained at a point somewhat less than halfway between the control and the two higher dosage lots throughout most of the experiment. Mortality in lot IV also differed significantly from the control after the fourth month of treatment ($P<0.005$ at 6 months) until the end of the experiment. During the last 4 months there was a marked increase in deaths in lot IV.

Differences between lots V and VI and the control were never statistically significant. However, by the end of the experiment deaths in lots V and VI exceeded those in the control by 20 percent and 37 percent, respectively. Most of this difference can be accounted for by high mortality in lots V and VI during two short periods centered about May and October 1961. These were periods of high mortality for all lots. It was found that many of the deaths in lots V and VI at these times resulted from failure of the fish to recover following release from the DDT baths. The control fish were held in aerated tanks during the administration of DDT to the bath-treated lots. Lots V and VI contained a greater total weight of fish at these times than did the other bath-treated lots. Attempts were made to keep the weight of fish per tank low enough to avoid losses from crowding. However, at the times mentioned above it appears that there were additional factors, such as reduced waterflow and handling, which caused unusual heavy losses at weight levels which had previously proved safe. As the residue levels of chlorinated hydrocarbons in samples from lots V and VI did not differ greatly from those found in the control, it is unlikely that DDT treatment was one of these factors. It is felt that these occasional excessive losses should be attributed primarily to the stress of crowding for short periods of time during treatment.

DDT in food.—Lot VII showed a sudden and marked reaction to treatment after only 1 month, and cumulative mortality in this lot differed significantly from the control at the end of 3 months ($P<0.05$). Most of the fish from lot VII which are included in the mortality data had died by the end of 7 months. At this time, mortality in this lot was more than 10 times that in the control. At the end of the experiment total mortality was still about 3½ times as great as in the control. All of the fish in lot VII not removed alive for samples had succumbed by April 1962, 3 months before the treatment of other lots was terminated.

Lot VIII evinced rapid mortality similar to lot VII about 3 months after treatment was begun, and its cumulative mortality differed significantly from that of the control following the fourth month ($P<0.01$). Mortality in lot VIII diminished at the end of the seventh month when it approached about three-quarters of the magnitude of that in lot VII. At this time, cumulative mortality in lot VII was significantly higher than that in lot VIII ($P<0.01$). However, subsequent mortality in lot VIII during the 15th through the 18th months brought total mortality for this group very close to the near total loss of fish exhibited by lot VII at this time.

Mortality in lots IX, X, and XI was almost identical to that in the control group throughout most of the experiment. Even the terminal value of lot IX was not significantly different from the control in spite of an appreciable increase in mortality during the 17th, 18th, and 19th months.

Relative mortality

Relative mortality within lots was calculated by comparing deaths per month with the average number of living fish in each lot during the month. The rates of mortality per month are expressed as percentages in figures 2 and 3. The

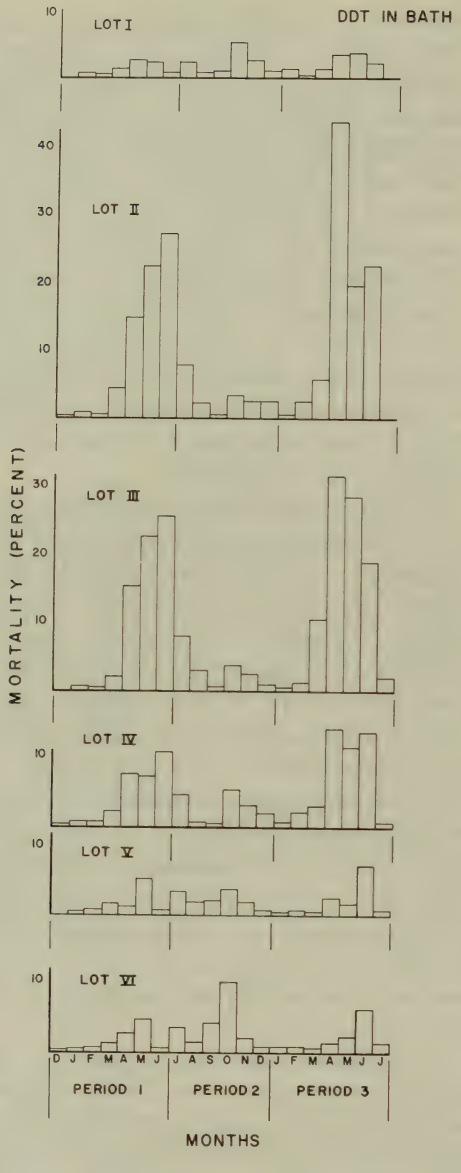


FIGURE 2.—Monthly mortality of fish receiving DDT in bath.

heavily dosed fish still had a high relative mortality toward the end of the experiment.

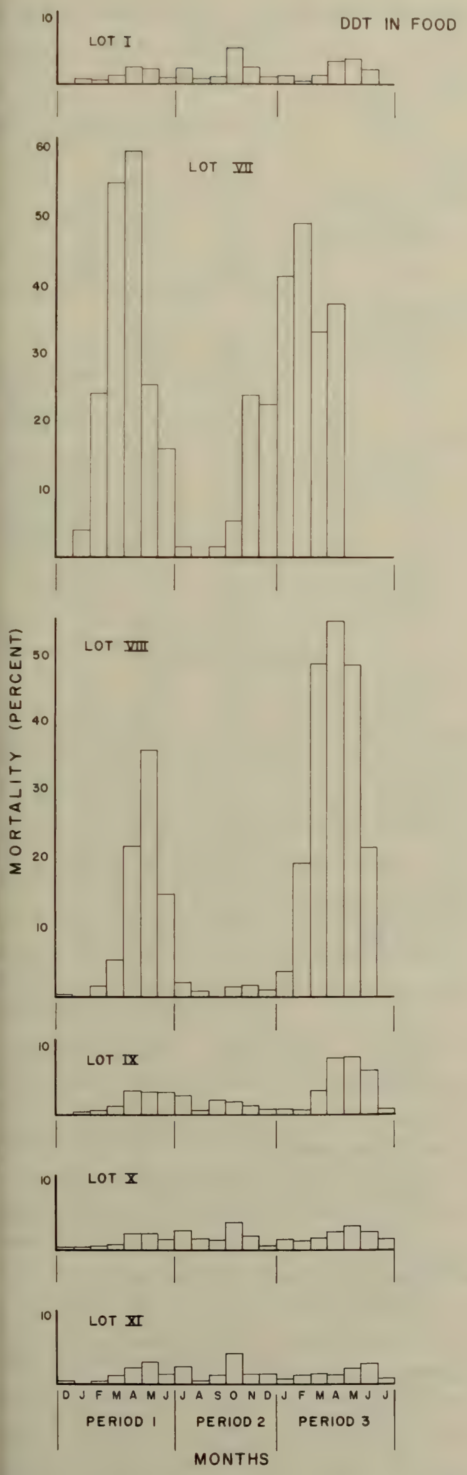
DDT in bath.—All of the bath-treated lots, as well as the control, displayed a trimodal distribution of relative mortality (fig. 2). These modes all fell near the 6th, 11th, and 18th months following initial treatment. The magnitude of relative mortality rates of lots II and III was similar. The highest dosage lots, II and III, also resembled each other in the low middle mode. The lower dosage lots and the control exhibited significant relative mortalities during this middle period. Mortality in lot IV paralleled that in lots II and III. Lots V and VI did not differ greatly from the control. Lot VI and the control both had their period of maximum relative mortality during the 11th month.

DDT in food.—Relative mortality rates in lot VII had a bimodal distribution in time (fig. 3). The first and higher mode occurred around the fourth and fifth months. A period of low relative mortality encompassed the 4th through 11th months. This was followed by high relative mortality until all of these fish had either died or been removed as samples by the latter part of the 17th month.

Mortality in lot VIII resembled the bimodal distribution of lot VII but occurred after a lag of 1 or 2 months. However, in this case, the second mode was greater than the first in magnitude and it closely approached the death rate of the first mode of lot VII during the previous year. As with the control and the bath-exposed fish, distribution of relative mortality rates in lots IX, X, and XI was trimodal. The first and last modes of lot IX were higher than the middle mode. Lot IX also reflected the conditions of lot VIII by displaying its highest mortality during the last few months of the experiment. The monthly death rates of lots X and XI were very similar to those of the control during the entire experiment.

Symptoms of disease

All fish which died were examined macroscopically for symptoms of disease and traumatic injury. Numbers and percentages of dead fish displaying obvious symptoms of disease at time of death (table 3) exhibited a trimodal distribution



IGURE 3.—Monthly mortality of fish receiving DDT in food

TABLE 3.—*Disease symptoms among dead fish*

Lot, treatment, and period [1]	Number of dead fish	Number of dead fish with symptoms of disease	Incidence of disease symptoms among dead fish
			Percent
Control:			
Lot I (no DDT):			
Period 1	34	21	62
Period 2	48	17	35
Period 3	41	21	51
Lot I	123	59	48
DDT in bath:			
Lot II (1.0 p.p.m.):			
Period 1	266	54	20
Period 2	42	13	31
Period 3	94	15	16
Lot II	402	82	20
Lot III (0.3 p.p.m.):			
Period 1	259	64	25
Period 2	42	4	10
Period 3	102	13	13
Lot III	403	81	20
Lot IV (0.1 p.p.m.):			
Period 1	131	34	26
Period 2	55	13	24
Period 3	98	19	19
Lot IV	284	66	23
Lot V (0.03 p.p.m.):			
Period 1	48	28	58
Period 2	61	11	18
Period 3	46	18	39
Lot V	155	67	37
Lot VI (0.01 p.p.m.):			
Period 1	49	24	49
Period 2	88	32	36
Period 3	35	16	46
Lot VI	172	72	42
DDT in food:			
Lot VII 3.0 mg./kg.):			
Period 1	396	60	15
Period 2	26	7	27
Period 3	27	5	18
Lot VII	449	72	16
Lot VIII (1.0 mg./kg.):			
Period 1	295	72	24
Period 2	14	2	14
Period 3	138	16	12
Lot VIII	447	90	20
Lot IX (0.3 mg./kg.):			
Period 1	55	25	45
Period 2	41	19	46
Period 3	86	21	24
Lot IX	182	65	36
Lot X (0.1 mg./kg.):			
Period 1	31	22	71
Period 2	53	22	42
Period 3	50	22	44
Lot X	134	66	49
Lot XI (0.03 mg./kg.):			
Period 1	42	30	71
Period 2	45	15	33
Period 3	35	16	46
Lot XI	122	61	50

[1] Period 1, Dec. 8, 1960, through June 30, 1961; period 2, July 1, 1961, through Dec. 31, 1961; period 3, Jan. 1, 1962, through July 31, 1962.

of relative mortality previously discussed. The relative incidence of disease symptoms among dead fish was much higher in the control and low-dosage lots than in the high-dosage groups (table 3). Conversely, the absolute number of disease-marked fish was highest in the high-dosage lots.

Sex ratios of mortalities

The data for the sex ratios of dead fish are given in table 4. In lots II, VII, and VIII nearly all of the fish had either died or been removed as samples by the end of the experiment, and their sex had been determined. The initial distribution of the sexes was fairly even in these three lots, and there is no reason to believe that this was not the case in the remaining lots.

In all lots except lot V, more males than females died during the first 7 months of the experiment. There was no evidence of selectivity due to differences in treatment levels. After some of the fish had died, as in the high-dosage lots, females would have outnumbered the males among the survivors. This must be kept in mind when examining the sex ratio data from the remaining periods. Since mortality of males in the control lot was accelerated to about the same degree as that of the treated fish, it appears that the toxicity of DDT was not selective for either sex.

Mortality among artificially spawned females

A record was kept during part of the 1962 spawning season on the number of females stripped of eggs in each lot and the number of these which died within the next few weeks. This information is presented in table 5.

Fish spawned from the high-dosage lots had larger average sizes and appeared to be in better physical condition than many of those spawned from the control and low-dosage lots. In spite of this, losses

TABLE 4.—*Sex ratios in fish dying during experiments*

Lot treatment, and period [1]	Number		Percent	
	Males	Females	Males	Females
Control:				
Lot I (no DDT):				
Period 1	22	12	65	35
Period 2	29	19	60	40
Period 3	23	18	56	44
Lot I	74	49	60	40
DDT in bath:				
Lot II (1.0 p.p.m.):				
Period 1	162	104	61	39
Period 2	16	26	38	62
Period 3	44	50	47	53
Lot II	222	180	55	45
Lot III (0.3 p.p.m.):				
Period 1	164	95	63	37
Period 2	16	26	38	62
Period 3	44	58	43	57
Lot III	224	179	56	44
Lot IV (0.1 p.p.m.):				
Period 1	82	49	63	37
Period 2	30	25	55	45
Period 3	49	49	50	50
Lot IV	161	123	57	43
Lot V (0.03 p.p.m.):				
Period 1	20	28	42	58
Period 2	33	28	54	46
Period 3	14	32	30	70
Lot V	67	88	43	57
Lot VI (0.01 p.p.m.):				
Period 1	30	19	61	39
Period 2	49	39	56	44
Period 3	12	23	34	66
Lot VI	91	81	53	47
DDT in food:				
Lot VII (3.0 mg./kg.):				
Period 1	222	174	56	44
Period 2	8	18	31	69
Period 3	10	17	37	63
Lot VII	240	209	53	47
Lot VIII (1.0 mg./kg.):				
Period 1	186	109	63	37
Period 2	5	9	36	64
Period 3	55	83	40	60
Lot VIII	246	201	55	45
Lot IX (0.3 mg./kg.):				
Period 1	34	21	62	38
Period 2	23	18	56	44
Period 3	54	32	63	37
Lot IX	111	71	61	39
Lot X (0.1 mg./kg.):				
Period 1	18	13	58	42
Period 2	31	22	58	42
Period 3	17	33	34	66
Lot X	66	68	49	51
Lot XI (0.03 mg./kg.):				
Period 1	27	15	64	36
Period 2	29	16	64	36
Period 3	12	23	34	66
Lot XI	68	54	56	4

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[1] Period 1, Dec. 8, 1960, through June 30, 1961; period 2, July 1, 1961, through Dec. 31, 1961; period 3, Jan. 1, 1962, through July 31, 1962.

following spawning were higher in stripped females from the high-dosage lots than from the low-dosage lots. The death rates of spawned females in the high-dosage lots were considerably higher than the death rates for the entire groups.

TABLE 5.—*Mortality among spawned females compared with mortality among remaining fish and with mortality for all fish*

[Data for period Feb. 21, 1962, to Apr. 30, 1962, for lots II and VIII and for period Feb. 21, 1962, to Apr. 12, 1962, for all other lots]

Lot	Treatment	Spawned females only			All fish except spawned females			All fish including spawned females		
		Number of fish	Number dying	Percent dying	Number of fish	Number dying	Percent dying	Number of fish	Number dying	Percent dying
Control: Lot I	0	39	4	10.3	333	8	2.4	372	12	3.2
DDT in bath:	*P.p.m.*									
Lot II	1.0	50	32	64.0	128	30	23.4	178	62	34.8
Lot III	.3	35	16	45.7	152	10	6.6	187	26	13.9
Lot IV	.1	37	4	10.8	236	7	3.0	273	11	4.0
Lot V	.03	39	2	5.1	331	3	.9	370	5	1.4
Lot VI	.01	39	2	5.1	284	2	.7	323	4	1.2
DDT in food:	*Mg./kg.*									
Lot VII	3.0	1	1	100.0	16	9	56.3	17	10	58.8
Lot VIII	1.0	23	20	87.0	128	76	59.4	151	96	63.6
Lot IX	.3	51	6	11.8	326	13	4.0	377	19	5.0
Lot X	.1	44	2	4.5	330	6	1.8	374	8	2.1
Lot XI	.03	33	1	3.0	319	5	1.6	352	6	1.7
All lots		391	90	23.0	2,583	169	6.5	2,974	259	8.7

DISCUSSION

All of the experimental lots, the control included, displayed peaks of mortality during spring and early summer of both years. None of the fish exhibited typical symptoms of acute DDT poisoning. However, the lots which received the most DDT had the highest death rates at these times. It can be surmised that DDT was acting in conjunction with other factors in producing mortality.

Another peak in mortality occurred in October 1961 in all lots except VII and VIII. This peak was relatively low in ots II and III. The control and lots VI, X, and XI suffered more losses than in any other month. The high-dosage lots had ower mortality rates than the control.

Some factors which may have affected mortality rates were temperature, disease, drugs administered for treatment of disease, and handling during the spawning period. Deaths sometimes followed the use of drugs, but there was no apparent correlation between such treatment and he periodic accelerations in mortality ates. Spawning in the spring of 1962 contributed to mortality; the heaviest losses were in the high-dosage lots.

Evidence of disease was prevalent among the dead fish (table 3). Throughout the experiment the low-dosage lots contained obviously sick fish which survived for extended periods of time but were the first to succumb during the periods of high mortality. Fish in the high-dosage lots displaying signs of illness seldom survived long. The second period noted in table 3 included the October 1961 peak in mortality of the control and low-dosage lots. During this period there was a low incidence of disease symptoms in all the fish, associated with low mortality rates in the high-dosage lots. The higher mortality in low-dosage and control lots during this period may reflect an additional stress factor operating on weaker fish, perhaps of a constitutional type which had largely been eliminated during the first period in the high-dosage lots. The periodicity of mortality in all lots, including the control, seems to indicate that DDT in the treated lots increased mortality in this experiment by reducing resistance to other, nonspecific, stresses.

GROWTH

Throughout most of the experiment the pens of fish were inventoried at intervals of approximately 4 weeks. The average weight of the fish in the individual sublots was used as a measure of growth and for calculation of the feeding schedule. Calculation of true growth was complicated by the removal of living fish for scheduled samples and by mortality between inventory dates. Thus, most increments in average weight between inventory dates are biased to some extent. The analysis which follows is based on apparent growth as derived from the average weight of surviving fish.

Weights from the three sublots for each treatment level have been grouped to give the average weight per lot used in the analysis. Differences between sublots within treatment levels were small. The average weights of treated lots were statistically compared to the control by *t* test throughout the experiment.

RESULTS

Apparent cumulative growth

Figure 4 illustrates the cumulative

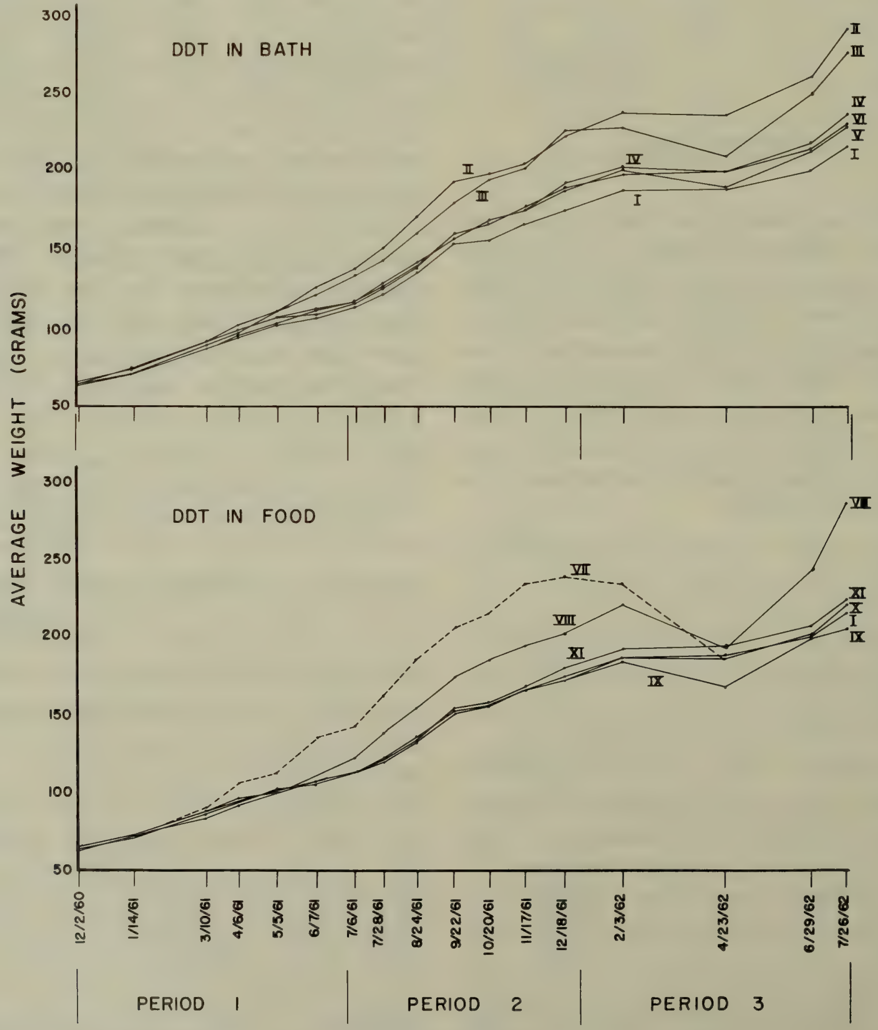

FIGURE 4.—Average weights of surviving fish. The dashed line representing the data for lot VII signifies the reduced numbers of fish present.

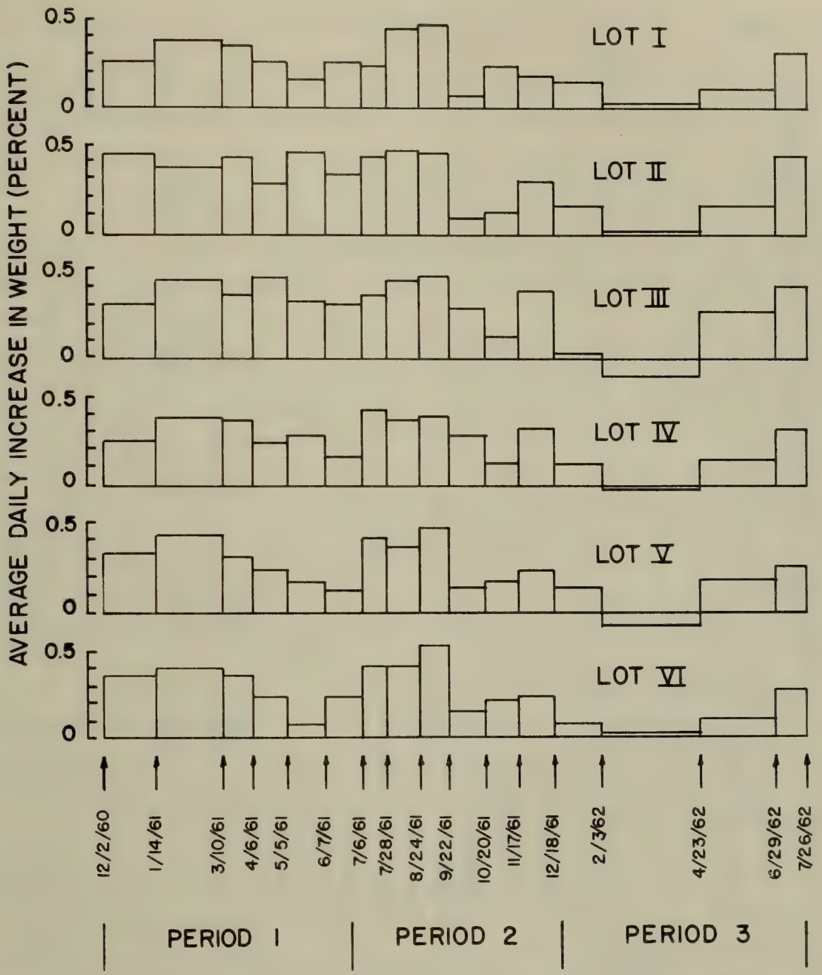

Figure 5.—Average daily weight changes in surviving fish receiving DDT in bath.

changes in average weight of surviving fish in each of the treatment lots. The average weights of the fish in lots II, III, VII, and VIII began to diverge from those of the other lots from February to May, and were significantly greater than the average of the control following 6 or 7 months of treatment. In statistical comparisons between these groups and the control, *t* values showed the differences to be significant ($P<0.025$). Values for the lower dosage groups did not generally differ significantly from the control; the few exceptions were confined to lots IV and V from December 1961 to February 1962.

Apparent growth rates

Average daily increases in weight between inventory dates, expressed as a percent of the average weight at the beginning of each period, are presented in figures 5 and 6. These values demonstrate that the apparent growth rates for short periods of time were quite similar for all treatment levels. Periodic fluctuations in apparent growth rate were reflected throughout all of the lots at approximately the same date.

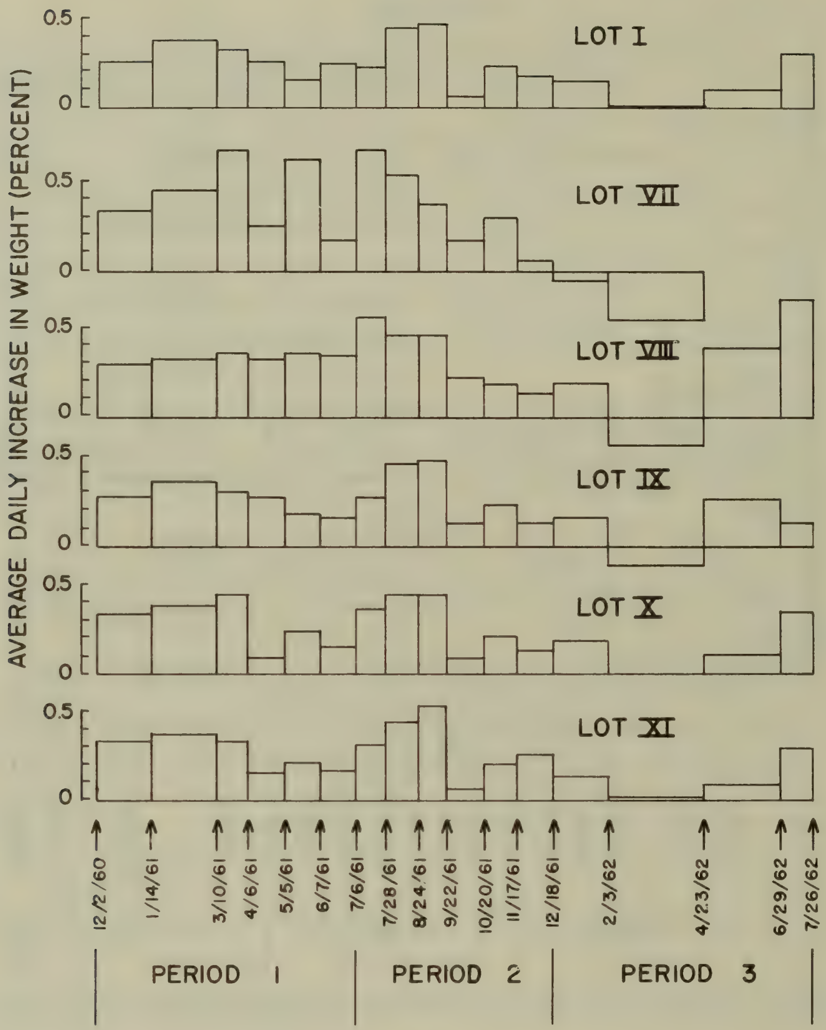

FIGURE 6.—Average daily weight changes in surviving fish receiving DDT in food.

DISCUSSION

Effects of sampling and mortality

Fish removed for scheduled samples were usually somewhat smaller than the average, and their removal tended to increase the apparent growth. However, after the initial 2 months, the numbers taken for this purpose were small. The total loss of fish from sampling was approximately the same for all lots throughout most of the experiment. Therefore, to simplify analysis, it may be assumed that bias from this source was applied equally at all treatment levels.

Weights of fish that died during the experiment were compared with the average weights of living fish at each inventory date. The samples included all dead fish collected daily for 4 days before, 4 days after, and on each inventory date. It is difficult to assess quantitatively the degree to which the high-dosage lots differed from low-dosage and control lots, since cumulative mortalities varied so markedly. It appeared, however, that dead fish of lot I did not differ in weight from the survivors of that lot, whereas throughout the first year in all treated lots the average weight of the dead fish was to some degree lower than that of the survivors. As the populations of the treated lots became depleted, the relative incidence of large dead fish increased. During the spawning season in the spring of 1962 the dead fish averaged slightly heavier than the survivors. As most of the ripe fish in any lot were also the largest fish, the brunt of the stress from handling at this time was borne by the heaviest individuals. However, dead fish in lots VII and VIII began to outweigh the survivors as early as December 1961, long before spawning operations were started.

Effects of spawning operation

During the spring of 1962, all lots were handled frequently and, in addition, they were not fed for extended periods before being spawned artificially. The effects of handling and starvation are illustrated in figures 4, 5, and 6 for the period February 3 through April 23, 1962, when apparent growth rates ranged from small positive to large negative values. As might have been expected, the fish became thin and most of the apparent decrease in growthrate for this period reflects the actual condition of the fish, including loss of body mass when eggs were extruded. In addition, the death of heavier-than-average fish at this time tended to reduce the apparent growth rate.

Immediately after spawning, all surviving lots showed some increase in average weight. However, mortality rates were high at this time, and growth values are considered to be strongly biased.

True growth

A sudden drop in mortality occurred after the end of June 1962. Some lots had no mortality and others lost from one to five fish. Since no samples were removed during the period June 29 through July 26, 1962, the increment of growth between these dates may be construed to approach a measure of the true growth rate at this time. The average weight and the number of fish in each lot did, however, vary considerably. All of the fish used in the experiment were the same age, but the high-dosage lots were composed predominantly of large fish which had demonstrated good growth up to this time. Therefore, it is not too surprising that these lots displayed the greatest percent increase, as shown in table 6. This suggests that growth is not inhibited in fish exposed to higher dosages of DDT.

Data for lot VII are absent from table 6. This lot was depleted through mortality and sampling some months earlier. Fortunately there was a 56-day period in the late summer of 1961 when lot VII suffered no mortality or losses due to sampling and the control was affected only slightly by these factors. As shown in table 7, percentage growth was almost identical in these lots at that time.

A search for an explanation why fish surviving exposure to the most DDT were the largest fish during most of the experiment considered the following possibilities: that it was a chance occurrence; that DDT killed the small fish, and spared the large ones; that DDT killed the diseased, slow-growing fish, and left the fast-growing ones; that DDT stimulated growth; and that a combination of the above factors was responsible.

Chance is ruled out as an explanation for the occurrence of larger fish in the heaviest treated groups. Figure 4 presents too consistent a picture of the relative positions of each group throughout the 20-month span.

Selection by the elimination of small fish in the high-DDT lots was probably

TABLE 6.—*Increase in weight of fish during period June 29 through July 26, 1962*

Lot	Treatment	Number of fish	Initial average weight (grams)	Increase in average weight (grams)	Percent increase in weight	
					Entire lot	Range for sublots
Control:						
Lot I	0	305	201	16	8.0	6.9–8.6.
DDT in bath:	*p.p.m.*					
Lot II	1.0	50	261	31	11.9	7.3–14.4.
Lot III	.3	48	250	27	10.8	7.3–29.1.
Lot IV	.1	145	219	18	8.2	6.4–9.3.
Lot V	.03	291	214	15	7.0	6.5–8.5.
Lot VI	.01	263	215	16	7.4	3.2–9.2.
DDT in food:	*mg./kg.*					
Lot VIII	1.0	16	244	43	17.6	1 sublot.
Lot IX	.3	263	200	6	3.0	0–6.3.
Lot X	.1	307	202	19	9.4	6.6–12.6.
Lot XI	.03	291	209	17	8.1	6.0–11.6.

TABLE 7.—*Increase in weight of fish in lots I and VII during the period July 28 through September 22, 1961*

Lot	Treatment	Number of fish	Initial average weight (grams)	Increase in weight (grams)	Percent increase in weight	
					Entire lot	Range for sublots
Lot I	0	458	121	32	26.4	25.4–28.0
Lot VII	3 mg./kg	60	163	44	27.0	25.6–30.7

a factor in this occurrence, although our data did not show significant differences which could be correlated to dosage. Selection by the elimination of low-vigor, slow-growing fish by DDT might also help explain what happened. It has been pointed out in the discussion of disease and survival that fish with visible pathology were more quickly killed if they had been exposed to large amounts of DDT than if the exposure had been slight. This suggests the possibility that the survivors in high-dosage lots grew faster than those in low-dosage lots, since the low-dosage lots had a higher incidence of disease and presumably had more fish of low vigor. It is assumed that disease was originally as prevalent in large fish as in small ones. Our data on disease do not reveal any relation between fish size and incidence of disease, so this mechanism remains a possibility for explaining differences in fish size from lot to lot.

This concept appears to be compatible with the growth and mortality data. The differences between lots in growth rates would be expected to be related to differences in mortality rates. Figures 2, 3, and 4 show that size of fish in lots II and III began to diverge from that of fish in other lots in March 1961; mortality exceeded that in other lots in March. Size in lot VII began to increase over the control in February, the same month in which mortality rates became significantly different from that of the control. Lot VIII had important increases in mortality in March, and the corresponding increase in average size of surviving fish appeared in the same month. This connection between increase in mortality and differences in size lends credence to the theory that highly dosed lots of fish were larger because of the rapid death of small or diseased slow-growing individuals.

The explanation for the occurrence of large fish in the high-dosage lots may lie in the selective death of smaller and weaker fish with less growth potential.

RESIDUES

METHODS

Residues of chlorinated hydrocarbons were determined by a paper chromatographic procedure which is a modification of the method described by Mitchell (1958). The procedure as used in this laboratory has been described in detail (Kallman et al., 1962). Two purification steps were taken in preparing extracts for chromatography. These were acetonitrile partition and sulfonation. The use of MgO for cleanup was avoided, since it decomposes DDT and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane), both of which were present in the fish.

Analyses were performed on aliquots of homogeneous blends of whole fish and anhydrous Na_2SO_4. In analyzing adult fish, 50 grams of tissue was present in the aliquots; when the fish became large enough, these determinations were performed in duplicate and the results were averaged. Fry samples and pooled organs or tissues were treated similarly to adult fish. It was necessary to homogenize eggs in petroleum ether: diethyl ether (9:1, v:v) in a blender; the solids were filtered off and reextracted by shaking in the same solvent mixture. A minimum of one adult fish from each sublot was analyzed at each sampling.

RESULTS

Fairly high residues were found in control fish. The commercial diet on which the fish were fed before the start of the experiment contained chlorinated hydrocarbons on the order of 0.8 p.p.m. Chlorinated hydrocarbons were present in the basic diet fed to all the fish in the experiment. The main source of this material was a grossly contaminated batch of cod-liver oil, which was replaced after having been used for about 1 year. Table 8 contains analytical data for the various components of the feed and the final mixture. Table 9 contains residue data for adult fish expressed as total chlorinated hydrocarbon in parts per million; figures 7 and 8 show these data plotted.

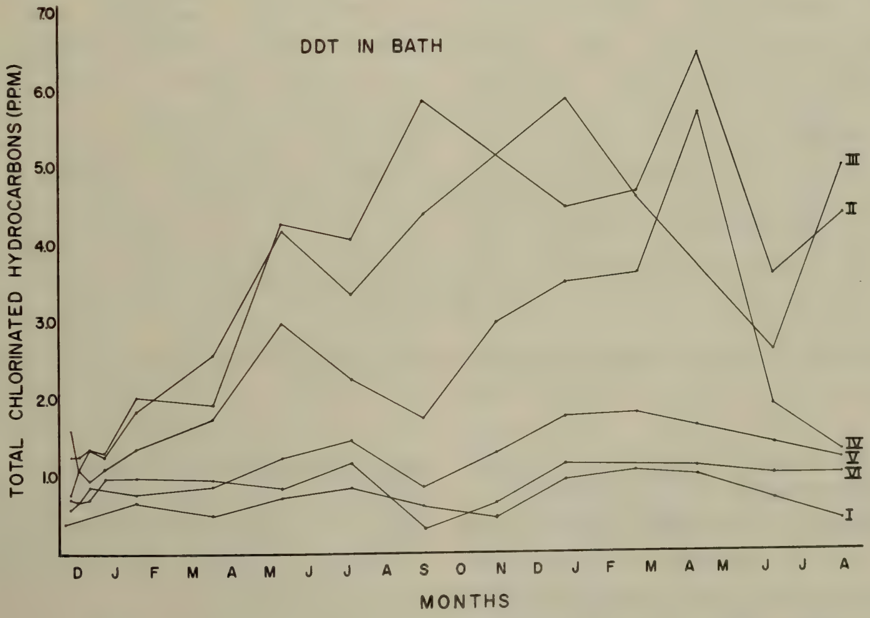

Figure 7.—Whole-body residues of chlorinated hydrocarbons in fish receiving DDT in bath.

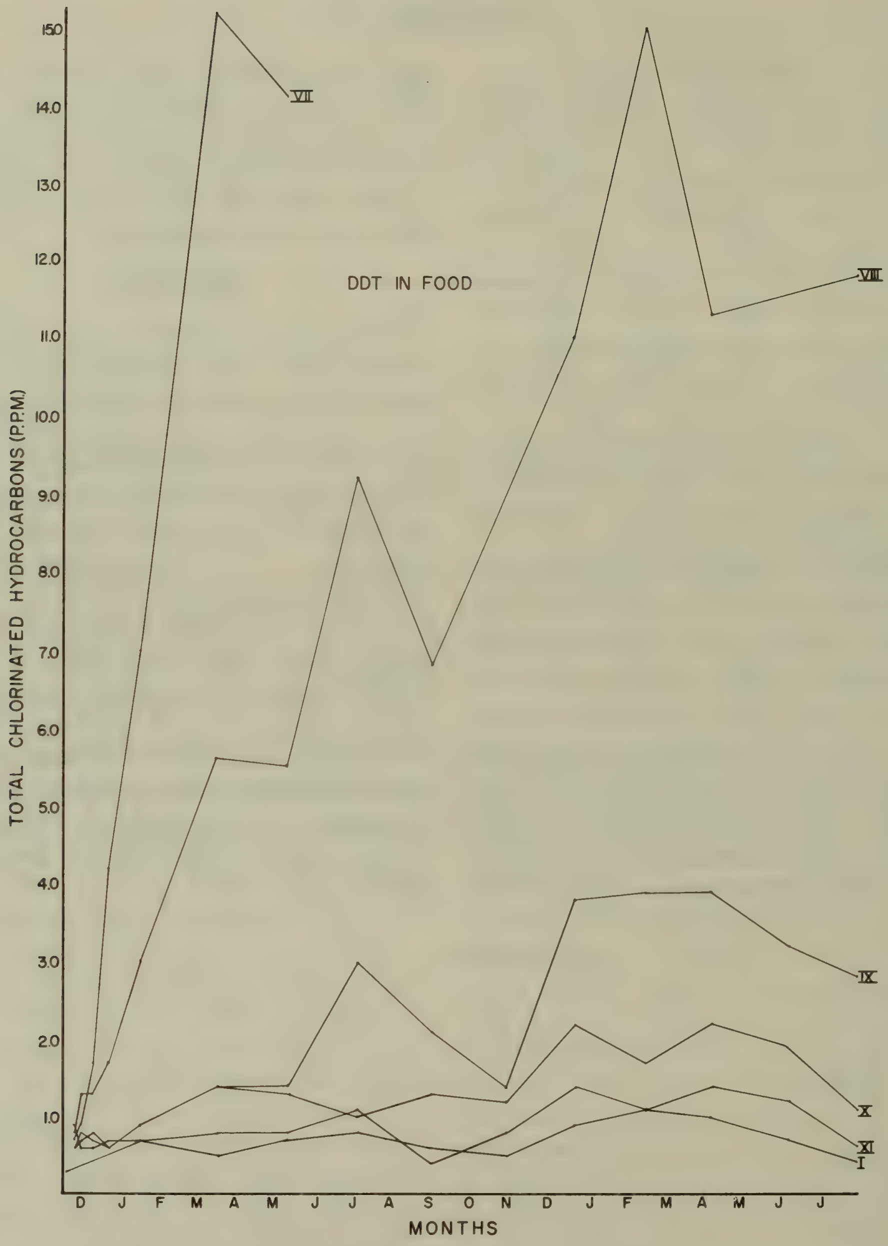

FIGURE 8.—Whole-body residues of chlorinated hydrocarbons in fish receiving DDT in food.

Residue data for eggs and fry are presented in table 10, and in table 11 for pooled organs and tissues of fish which died. The fish included in these pools died within the period November 1961 to March 1962. Table 12 shows residue data of adult fish in terms of relative amounts of the three chlorinated hydrocarbons found, DDT, DDD, and DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene).

TABLE 8.—*Concentrations of chlorinated hydrocarbons in components of the basic ration*

[Parts per million. ND=None detectable]

Component	DDT	DDE	DDD	Total
Mixture fed	0.15	0.46	0.15	0.76.
Cod-liver oil	3.0	17.0	3.7	23.7.
Molasses	ND	ND	ND	ND.
Skim milk	ND	ND	0.04	0.04.
Wheat middlings	ND	ND	Trace	Trace.
Whitefish meal	Trace	Trace	0.05	0.05+.
Yeast	Trace	Trace	Trace	Trace.
Cottonseed meal	0.03	0.04	Trace	0.07+.

DISCUSSION

Examination of the standard deviations presented in table 10 provides an indication of the extreme individual variation encountered in residue analysis for chlorinated hydrocarbons. This was found with both bath and feeding exposures. Despite this variation, definite trends can be found in the residue data. Considering the bath-treated fish, persistently high residues were found in lots II and III. These two lots did not differ significantly from each other in either mortality or residue buildup; this indicates that at concentrations of about 0.3 p.p.m. maximum uptake of DDT occurs, under the conditions of this experiment. Both lots appeared to reach a plateau of residue buildup; lot II did so somewhat earlier than lot III. Lot IV accumulated chlorinated hydrocarbon at a slower rate and continued to build up in residue throughout most of the study. Until late in the experiment, its residue amounts were distinct from those of lots receiving higher and lower treatment levels. Lot VI was almost identical to the control fish in amount of residues. Lot V was significantly different, by *t* test, from the control group at various times, but not at other times. This probably resulted from individual variation and the relatively small number of fish analyzed at each sampling; it seems extremely unlikely that this effect, or the crossing over of the residue curves of lots II and III, represents real biological events. Both exposures of 0.01 and 0.03 p.p.m. (lots VI and V) thus appeared to produce residue buildups not appreciably different from the controls, indicating an approximate equilibrium between accumulation and metabolism and excretion.

More distinct differences were observed in the lots receiving DDT in their feed than in those bathed in DDT. Residue accumulation in lots VII and VIII proceeded at rapid and different rates. There appears to be no limit to the initial rate of DDT uptake from food with the dosages used in this study, except that imposed by death. During the latter stages of the experiment, residues did level off or even drop somewhat in these two lots. A possible explanation for this might be an increased rate of metabolism and excretion. There did not appear to be a reduction in the amount of body fat in any of the DDT-exposed fish; presumably such a loss would lower the ability of the body to store chlorinated hydrocarbons. It is also possible that this apparent reduction in the rate of residue buildup occurred as a result of the heavy mortality in these two lots. Unfortunately, residue analyses were not performed routinely on samples of fish which died during the course of the experiment; possibly such fish contained significantly higher residues than the survivors, thus weighting future sampling toward lower results. Work in this laboratory has not indicated that fish which died during various LC_{50}[3] tests had higher residues of the test chlorinated hydrocarbons than did survivors.

Residues in lot IX were markedly lower than those of lot VIII, but higher than control levels. Lots X and XI were not significantly different from the controls.

No correlation was found between residue levels and either size or sex of fish in any of the lots.

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[3] LC_{50} is the effective concentration at which 50 percent of the test animals die.

TABLE 9.—*Mean whole-body residues of total chlorinated hydrocarbons*

[Parts per million. Numbers in parentheses represent numbers of fish analyzed]

	Lot I (control)	DDT in bath					DDT in food				
		Lot II	Lot III	Lot IV	Lot V	Lot VI	Lot VII	Lot VIII	Lot IX	Lot X	Lot XI
Sample 1	0.4±0.2(12)	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
Sample 2	----------	1.2±0.2(3)	1.6±0.2(3)	0.8±0.1(3)	0.7±0 (3)	0.6±0 (3)	0.7±0.3(4)	0.8±0.1(3)	0.6±0.1(4)	0.6±0.1(3)	0.9±0.1(5)
Sample 3	----------	1.2 .2(3)	1.1 .1(3)	1.1 .2(3)	.7 .2(4)	.7 .1(3)	.9 .2(3)	1.3 .4(3)	.8 .1(3)	.7 .2(3)	.6 .1(3)
Sample 4	----------	1.4 .1(3)	1.3 .4(4)	1.0 .1(3)	.9 0 (3)	.7 .2(4)	1.7 .8(3)	1.3 .3(4)	.7 0 (3)	.8 .2(4)	.6 0 (3)
Sample 5	----------	1.3 .1(3)	1.3 .2(4)	1.1 .4(3)	----------	1.0 .2(3)	4.2 2.8(3)	1.7 .3(3)	.6 .3(3)	.6 .1(3)	.7 .1(3)
Sample 6	.7 .2(3)	2.0 .1(3)	1.8 .4(3)	1.4 .2(3)	.8 .1(3)	1.0 .2(3)	7.0 1.5(3)	3.0 .8(3)	.9 .4(4)	.9 0 (3)	.7 .1(3)
Sample 7	.5 .1(3)	1.9 .2(3)	2.6 .7(5)	1.8 .5(5)	.9 .1(3)	.9 .2(4)	15.2 3.5(5)	5.6 1.0(3)	1.4 .4(3)	1.4 .2(3)	.8 .2(3)
Sample 8	.7 .1(3)	4.3 .5(5)	4.2 .2(3)	3.0 .1(3)	1.2 .5(5)	.8 .1(3)	14.1 4.8(5)	5.5 2.2(6)	1.4 0 (3)	1.3 .2(3)	.8 .1(3)
Sample 9	.8 .2(6)	4.1 .5(5)	3.4 1.2(3)	2.3 .1(3)	1.5 .1(4)	1.2 .2(4)	----------	9.2 1.7(5)	3.0 1.9(5)	1.0 .7(3)	1.1 .1(3)
Sample 10	.6 .2(6)	5.9 .6(3)	4.4 .3(3)	1.7 .5(3)	.9 .3(6)	.3 .1(6)	----------	6.8 .6(4)	2.1 .7(3)	1.3 0 (3)	.4 .1(4)
Sample 11	.5 .2(6)	----------	----------	3.0 .7(4)	1.3 .7(4)	.6 .1(4)	----------	----------	1.4 .4(5)	1.2 .6(4)	.8 0 (4)
Sample 12	.9 .2(6)	4.5 .5(5)	5.9 .5(4)	3.5 .7(4)	1.8 .4(5)	1.2 .1(4)	----------	11.0 3.4(6)	3.8 2.1(6)	2.2 .4(6)	1.4 .3(5)
Sample 13	1.1 .4(6)	4.7 1.2(5)	4.6 .3(4)	3.6 .2(4)	1.8 1.3(4)	----------	----------	15.0 2.5(4)	3.9 1.5(4)	1.7 .7(6)	1.1 .2(4)
Sample 14	1.0 .1(6)	6.5 4.7(6)	3.7 1.3(4)	5.7 .6(4)	1.6 1.1(4)	1.1 .2(4)	----------	11.3 2.7(4)	3.9 1.8(4)	2.2 .7(4)	1.4 .5(4)
Sample 15	.7 .2(6)	3.6 1.6(6)	2.6 1.4(4)	1.9 .7(4)	1.4 .1(4)	1.0 .1(4)	----------	----------	3.2 1.0(4)	1.9 .4(4)	1.2 1.0(4)
Sample 16	.4 .1(6)	4.4 1.2(4)	5.0 1.0(5)	1.3 .6(4)	1.2 .2(4)	1.0 .2(4)	----------	11.8 1.5(4)	2.8 .9(4)	1.1 .5(4)	.6 .3(4)

TABLE 10.—*Residues of total chlorinated hydrocarbons in eggs and fry*

[Parts per million]

Lot and sublot	Treatment of adults	Eggs		Fry	
		"Pick-offs" collected about 4 weeks after spawning	Unfertilized	Collected 2 to 3 weeks after hatching	Collected 5 to 6 weeks after hatching
Lot I: Sublot 2	0	1.0	0.2	1.6	0.2
	p.p.m.				
Lot II	1.0	---	---	---	2.2
Lot III:					
Sublot 1	.3	3.2	2.5	3.9	[1] .7
Sublot 3	.3	4.5	2.3	6.0	
Lot IV:					
Sublot 1	.1	7.4	1.2	2.4	[1] 1.2
Sublot 3	.1	5.2	2.9	4.2	
Lot V:					
Sublot 1	.03	.7	1.4	3.1	---
Sublot 2	.03	1.8	---	---	.4
Sublot 3	.03	2.9	1.0	---	---
Lot VI	.01	---	---	---	.8
Lot VIII:					
	mg./kg.				
Sublot 1	1.0	.7	11.0	---	---
Sublot 3	1.0	5.3	19.8	13.2	---
Lot IX:					
Sublot 2	.3	3.0	5.8	5.9	[1] .9
Sublot 3	.3	3.9	3.1	2.8	
Lot X:					
Sublot 2	.1	1.1	2.4	2.1	[1] .6
Sublot 2	.1	.7	---	---	
Lot XI	.03	---	---	---	.2

[1] These figures represent analytical results of pooled samples from 2 sublots.

TABLE 11.—*Residues of total chlorinated hydrocarbons in tissues of dead fish*

[Parts per million. Numbers of fish are approximate]

Tissue	Lot II (8 fish)				Lots V and VI combined (15 fish)				Lot VIII (8 fish)				Lots X and XI combined (15 fish)			
	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total	DDT	DDE	DDD	Total
Brain	6.2	6.3	1.0	13.5	ND	1.3	ND	1.3	28.0	7.0	1.0	36.0	0.5	0.5	Trace	1.0
Skeletal muscle	3.3	1.5	.3	5.1	0.4	.3	0.1	.8	7.8	2.3	.8	10.9	.3	.3	0.05	.6
Liver	1.3	1.8	2.5	5.6	.2	.8	.4	1.4	1.8	1.4	7.0	10.2	.3	.9	.4	1.6
Ovary	8.6	2.9	.8	12.2	1.4	1.8	.3	3.5	4.8	.9	.9	6.6	.8	.7	.2	1.7
Gut	1.0	2.4	1.3	4.7	Trace	1.5	.4	1.9	1.0	.8	2.5	4.3	1.5	5.5	.3	7.3
Testes	.6	1.0	.4	2.0	.3	3.5	.1	3.9	1.3	.7	.4	2.4	.1	.2	.1	.4

TABLE 12.—*Percentage of total chlorinated hydrocarbons as DDT, DDE, and DDD in whole-body residues* [mean percent; range in parentheses]

A. DDT IN BATH

Sample No.—	Lot I			Lot II			Lot III			Lot IV			Lot V			Lot VI		
	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD
2	12 (5–23)	70 (62–87)	17 (6–29)	65 (60–70)	31 (28–35)	4 (3–5)	65 (58–71)	26 (21–32)	9 (8–10)	45 (41–50)	50 (44–55)	5 (3–6)	38 (38–39)	55 (53–56)	8 (7–9)	23 (19–25)	63 (60–68)	14 (13–16)
3	---	---	---	56 (53–60)	36 (33–38)	8 (7–9)	49 (46–55)	42 (38–46)	8 (7–9)	46 (39–50)	47 (42–55)	7 (6–8)	34 (26–43)	55 (51–59)	11 (6–16)	23 (19–28)	65 (64–66)	12 (8–16)
4	---	---	---	54 (53–55)	37 (36–39)	9 (8–9)	54 (49–58)	40 (37–45)	6 (3–10)	51 (49–53)	42 (37–45)	7 (6–10)	42 (38–48)	48 (42–55)	10 (7–13)	18 (6–32)	66 (58–71)	16 (10–22)
5	---	---	---	48 (43–50)	45 (43–49)	7 (6–8)	48 (43–54)	45 (41–50)	7 (5–11)	44 (42–45)	45 (42–49)	12 (8–13)	---	---	---	12 (11–13)	80 (75–81)	9 (6–12)
6	16 (8–20)	69 (65–75)	15 (13–17)	61 (59–63)	34 (32–36)	5 (5)	57 (46–65)	38 (31–48)	6 (4–7)	46 (41–52)	47 (41–50)	8 (7–9)	23 (18–26)	71 (68–75)	6 (5–7)	25 (16–30)	64 (57–74)	11 (9–13)
7	19 (12–29)	72 (60–80)	9 (8–10)	61 (55–64)	36 (32–42)	3 (2–4)	67 (56–76)	30 (21–41)	3 (2–3)	61 (52–76)	35 (23–44)	4 (1–7)	27 (25–29)	65 (59–72)	8 (4–12)	25 (18–35)	65 (50–74)	8 (8–15)
8	20 (15–23)	72 (67–78)	8 (6–9)	73 (71–77)	23 (20–26)	3 (2–5)	77 (72–80)	20 (17–25)	3 (2–3)	66 (59–70)	31 (27–38)	3 (3–4)	49 (26–63)	46 (32–67)	6 (5–7)	34 (30–37)	58 (57–59)	8 (6–11)
9	15 (7–28)	74 (60–82)	10 (7–14)	72 (67–75)	22 (19–27)	5 (4–6)	76 (65–84)	23 (15–34)	1 (1–2)	60 (54–66)	36 (28–44)	3 (1–5)	56 (53–62)	38 (36–43)	6 (2–9)	40 (30–45)	53 (45–64)	7 (5–10)
10	21 (6–44)	72 (51–87)	8 (5–15)	70 (65–78)	25 (18–30)	5 (4–6)	73 (70–77)	23 (19–27)	4 (3–4)	78 (72–84)	19 (14–24)	3 (2–4)	54 (36–65)	39 (29–54)	7 (3–12)	42 (33–50)	46 (43–55)	12 (7–14)
11	13 (4–20)	80 (73–90)	7 (6–8)	---	---	---	---	---	---	64 (51–74)	34 (22–47)	3 (2–4)	54 (48–60)	42 (36–49)	4 (3–4)	20 (15–30)	74 (62–80)	6 (5–8)
12	11 (6–13)	85 (82–90)	4 (4–6)	73 (66–79)	25 (20–32)	2 (1–2)	81 (80–82)	17 (14–18)	2 (2–4)	71 (65–76)	27 (22–32)	2 (1–3)	45 (39–52)	52 (44–58)	3 (2–4)	28 (27–29)	68 (68–69)	3 (2–4)
13	14 (11–17)	81 (78–84)	4 (2–7)	71 (64–79)	26 (19–33)	3 (2–4)	70 (66–74)	27 (23–32)	2 (2–3)	68 (67–68)	30 (29–32)	2 (1–4)	39 (34–45)	55 (49–61)	6 (4–10)	---	---	---
14	10 (4–13)	76 (73–81)	14 (10–19)	60 (47–79)	36 (15–49)	5 (2–8)	52 (33–65)	41 (34–53)	7 (1–13)	40 (30–46)	56 (47–67)	4 (2–7)	49 (41–53)	47 (42–54)	4 (0–6)	30 (14–38)	59 (48–82)	10 (4–15)
15	34 (12–58)	59 (34–88)	8 (0–32)	62 (48–72)	36 (24–52)	2 (0–6)	60 (48–70)	40 (30–52)	---	50 (38–62)	50 (38–62)	---	38 (33–50)	48 (34–57)	14 (11–17)	30 (25–36)	58 (47–68)	12 (7–17)
16	10 (3–20)	83 (73–92)	6 (0–10)	76 (68–83)	20 (14–29)	3 (3–4)	75 (68–81)	22 (16–28)	4 (3–6)	49 (32–65)	47 (30–65)	4 (2–6)	49 (42–59)	46 (38–53)	6 (3–7)	44 (35–49)	49 (42–60)	7 (–10)

B. DDT IN FOOD

Sample No.—	Lot I			Lot VII			Lot VIII			Lot IX			Lot X			Lot XI		
	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD	DDT	DDE	DDD
2	12 (5–23)	70 (62–87)	17 (6–29)	29 (7–50)	54 (37–71)	18 (13–27)	38 (29–52)	54 (40–64)	8 (7–10)	14 (5–23)	69 (65–76)	17 (13–23)	20 (17–22)	67 (61–72)	13 (11–17)	4 (0–4)	87 (84–89)	12 (9–12)
3	----------	----------	----------	34 (30–39)	56 (54–60)	10 (6–13)	45 (33–63)	47 (31–58)	7 (6–9)	25 (23–27)	68 (66–70)	7 (7–8)	26 (22–34)	59 (51–65)	15 (14–16)	24 (23–26)	63 (62–64)	14 (13–15)
4	----------	----------	----------	60 (57–64)	32 (30–36)	7 (5–10)	46 (31–55)	44 (36–45)	9 (8–11)	22 (19–24)	67 (65–69)	11 (7–16)	20 (15–30)	66 (56–74)	15 (14–16)	29 (25–32)	61 (58–65)	10 (9–10)
5	----------	----------	----------	83 (77–87)	13 (10–18)	3 (2–5)	64 (59–74)	27 (18–31)	9 (8–10)	37 (37)	53 (51–55)	9 (8–11)	24 (18–32)	66 (59–71)	10 (9–11)	21 (17–27)	67 (63–69)	13 (10–15)
6	16 (8–20)	69 (65–75)	15 (13–17)	88 (86–90)	10 (8–12)	2 (1–3)	69 (58–77)	26 (19–37)	5 (4–5)	25 (7–40)	62 (43–76)	13 (8–17)	21 (14–32)	68 (56–75)	11 (9–12)	21 (18–26)	65 (58–72)	14 (11–17)
7	19 (12–29)	72 (60–80)	9 (8–10)	85 (83–88)	12 (9–14)	3 (2–4)	81 (72–87)	15 (10–24)	3 (3)	49 (45–53)	45 (43–49)	6 (5–8)	40 (34–46)	49 (43–55)	11 (11–12)	35 (30–38)	53 (46–56)	15 (14–16)
8	20 (15–23)	72 (67–78)	8 (6–9)	82 (66–91)	14 (6–31)	3 (2–4)	69 (34–84)	28 (11–63)	4 (3–6)	37 (36–40)	59 (57–61)	4 (3–6)	58 (57–60)	35 (33–36)	7 (6–8)	44 (37–51)	47 (42–53)	9 (8–10)
9	15 (7–28)	74 (60–82)	10 (7–14)	----------	----------	----------	85 (83–88)	13 (9–15)	1 (0–3)	60 (52–65)	36 (31–45)	4 (3–5)	52 (42–60)	41 (29–53)	7 (5–10)	45 (43–48)	47 (42–50)	9 (6–11)
10	21 (6–44)	72 (51–87)	8 (5–15)	----------	----------	----------	91 (88–94)	6 (4–8)	3 (2–4)	82 (80–85)	14 (12–15)	4 (3–5)	63 (62–63)	33 (32–34)	4 (3–5)	51 (37–70)	39 (11–58)	10 (3–19)
11	13 (4–20)	80 (73–90)	7 (6–8)	----------	----------	----------	----------	----------	----------	59 (47–74)	39 (24–49)	2 (1–4)	45 (36–57)	52 (41–59)	3 (3–4)	36 (30–40)	58 (55–65)	5 (4–5)
12	11 (6–13)	85 (82–90)	4 (4–6)	----------	----------	----------	90 (89–91)	9 (8–10)	1 (1–2)	60 (27–74)	36 (23–60)	4 (2–13)	56 (50–60)	41 (37–47)	3 (2–3)	29 (19–43)	67 (51–76)	4 (3–6)
13	14 (11–17)	81 (78–84)	4 (2–7)	----------	----------	----------	86 (83–90)	11 (8–15)	2 (1–5)	68 (54–77)	29 (21–43)	3 (2–5)	53 (24–67)	44 (29–74)	2 (2–4)	31 (18–40)	62 (52–77)	6 (4–9)
14	10 (4–13)	76 (73–81)	14 (10–19)	----------	----------	----------	74 (67–80)	22 (14–32)	4 (1–6)	60 (43–78)	35 (18–51)	5 (4–7)	55 (51–63)	38 (30–43)	7 (6–9)	39 (27–48)	57 (51–67)	4 (2–6)
15	34 (12–58)	59 (34–88)	8 (0–32)	----------	----------	----------	----------	----------	----------	60 (55–65)	30 (28–35)	10 (6–11)	53 (46–62)	40 (33–47)	6 (5–8)	40 (21–66)	57 (31–79)	4 (0–10)
16	10 (3–20)	83 (73–92)	6 (0–10)	----------	----------	----------	80 (77–82)	17 (14–20)	2 (2–3)	65 (58–76)	31 (20–40)	3 (2–4)	42 (24–62)	53 (33–70)	5 (4–6)	23 (17–26)	70 (65–83)	6 (0–11)

REPRODUCTION

METHODS

During the spring of 1962, about 15 months after the experiment was begun, many of the fish approached sexual maturity. Eggs were stripped from females as they became ripe through a period of more than 3 months. While lack of sufficient hatching facilities prevented the incubation of all of the eggs, an attempt was made to rear at least three collections from each of the three sublots representing each treatment level. In some cases, where few eggs were taken each time, as many as six collections were incubated from a single sublot. Eggs were fertilized with milt from males in the same sublot. All collections were kept segregated by sublot and date of spawning.

For each collection (i.e., eggs from one sublot taken on a given day) the volume of eggs was determined by displacement. The average size of the eggs in each collection was determined by sample count in a standard measuring trough.

After measurement for egg production, coagulated eggs were removed and measured to determine initial mortality. The remaining eggs were then incubated in hatching jars modified as outlined by Buss (1959). Coagulated and undeveloped eggs were removed and measured volumetrically following eyeing and hatching.

The surviving fry were raised to various stages of development in the hatching jars. Most of the fry were sacrificed for samples a few weeks after hatching. However, some of them representing most of the treatment levels were still alive and growing well more than 8 months after the eggs were fertilized.

RESULTS

Sexual development of adults

The proportion of sexually mature fish was higher in the high-dosage lots than in the low-dosage groups. Approximate percentages of females which were ripe when fish were spawned were as follows: I, 4; II, 7; III, 8; IV, 6; V, 4; VII, 10; VIII, 7; IX, 5; X, 3; XI, 4. This was apparently due to the higher incidence of small fish in the control and low-dosage lots. Subjective observation at time of spawning revealed no discernible differences in sexual development between fish of like size at any of the treatment levels.

Egg production

In all lots there were wide variations in both the size and the number of eggs produced by individual females. When these data were grouped by lot, the average egg production was similar for most treatment levels (table 13). The volume and numbers of eggs produced by lot VIII fish stands out higher than the rest, but it, along with lot III, contained larger-than-average females at this time. Moreover, the volume of eggs per unit weight of female was also highest in lots III and VIII. In any event, prolonged treatment with DDT at the higher levels apparently was not detrimental to numbers and volumes of eggs produced in the survivors.

Development of progeny

Mortality rates for developing eggs are presented in table 14. Considering the environmental factors involved, the survival rates for all lots during incubation were uniform. The notable exception was lot VII, the fish fed the most DDT, where only one female survived for spawning. Low survival in this case may have been caused by DDT or may simply reflect poor fertilization or some individual deficiency in one of the parents.

Mortality among the sac-fry of all lots was so high that it proved impractical to make accurate measurements of losses at this stage. However, the mortality of

TABLE 13.—*Egg production of artificially spawned fish*

	Treatment	A—Group of females				B—Random sample from A		
		Number of females	Milliliters of eggs per female	Estimated number of eggs per female [1]	Number of eggs per milliliter	Number of females	Average weight in grams	Milliliters of eggs per kilogram of fish
Lot I	0	45	30.7	870	28.3	15	200	172
	p.p.m.							
Lot II	1.0	52	35.8	1,000	27.8	35	201	179
Lot III	.3	37	38.1	1,030	27.1	18	221	206
Lot IV	.1	40	34.0	940	27.7	21	214	175
Lot V	.03	46	33.8	930	27.5	19	214	165
Lot VI	.01	41	33.4	880	26.2	24	201	178
	mg./kg.							
Lot VII	3.0	1	32.0	860	26.9	1	260	123
Lot VIII	1.0	25	43.8	1,210	27.7	18	227	197
Lot IX	.3	50	33.1	920	27.7	27	204	175
Lot X	.1	34	30.6	870	28.5	21	212	164
Lot XI	.03	41	35.2	1,000	28.3	25	200	184

[1] Rounded to the nearest 10.

sac-fry was discernibly higher in lots II, III, and VIII than in lots I, IV, V, VI, IX, X and XI. Only five eggs hatched in lot VII, and these fry died within a few days. Very few fry from lot VIII survived until the feeding stage; sac-fry mortality in lots II and III was not as high. Once the fry began to feed, mortality was low in all of the lots, including the few survivors of lot VIII. The growth rates for individual collections of fry were variable but there was no apparent correlation with the exposures their parents had received. Residue data are presented in table 11.

TABLE 14.—*Mortality rates of eggs*

[Percentages are based on eggs remaining after previous pickoff]

Lot	Treatment of adults	Initial volume of eggs	Mortality after hardening	Mortality between initial pickoff and eyeing	Mortality between eyeing and hatching
		Milliliters	*Percent*	*Percent*	*Percent*
I	0	1,381	3.3	53.1	10.0
	P.p.m.				
II	1.0	1,861	.7	48.7	10.5
III	.3	1,411	1.8	40.2	13.6
IV	.1	1,360	.8	42.0	9.4
V	.03	1,553	2.0	32.9	9.5
VI	.01	1,370	2.9	34.4	11.0
	Mg./kg.				
VII	3.0	32	19.3	90+	90+
VIII	1.0	1,095	3.1	47.6	8.5
IX	.3	1,653	2.5	41.8	11.0
X	.1	1,060	.9	41.4	19.1
XI	.03	1,445	1.9	38.5	9.4

DISCUSSION

There was no indication that DDT at the levels administered in this experiment affected the rate of sexual development or gamete production of the treated adults. Fertilized eggs from treated adults developed as quickly as those from the controls, and their survival to hatching was even somewhat better. However, there was a critical period shortly after hatching when mortality was noticeably higher in offspring of the high-dosage lots. Losses were heavy in all lots at this time. Therefore, excessive mortality in some groups might indicate a lower resistance to stress. On the other hand, these high losses could conceivably be due to absorption of yolk material containing high residue levels of DDT and its metabolites.

The residue data for eggs and fry showed that appreciable amounts of chlorinated hydrocarbon are passed along by the parent fish. The results were highly variable (in one case, almost a tenfold difference existed in residue of eggs collected from two sublots receiving the same treatment), but in general, higher residues were found in the eggs of fish receiving higher dosages of DDT. The relative amounts of DDT, DDE, and DDD did not appear to differ from those in adult fish whole-body residues. No significant pattern could be distinguished in residue levels of nonviable eggs as compared to those of unfertilized eggs, which presumably consisted of a mixture of the nonviable and viable. Residues in yolk-sac fry were somewhat higher than those in eggs from comparable lots; since no additional DDT was provided during development, this apparent increase probably resulted from variation in the samples analyzed. The lower concentrations present in later fry samples may represent a selection of a population with low residues or might result from metabolism or excretion of the chlorinated hydrocarbons.

By the time the fry were ready to accept food the daily mortality was substantially the same in all lots.

Survivors of all lots displayed equal growth and vigor during an additional 6 months of observation. However, in the case of the high-dosage lots, these survivors represented only a small portion of the reproductive potential of their parental stock when compared with the survival rate of the control progeny.

RESISTANCE TO STRESS

METHODS

An attempt was made to determine whether exposure to DDT reduced resistance to an experimentally imposed stress. Unfortunately, time was very short because of an impending sterilization of the raceways. Investigation showed that, with the available equipment, conditions could be controlled best in an experiment designed to test the ability of treated fish to withstand conditions of hyperosmolarity. The following procedures were adopted after preliminary tests to determine concentrations and exposure periods.

Test fish were taken from lots randomly, but injured or weak individuals were discarded. Treatment was carried out on approximately 20 fish at a time (14 minimum, 21 maximum) in 100 liters of aerated water containing 4 percent NaCl by weight. The temperature was maintained at 50° F., and dissolved oxygen was kept at saturation level throughout the treatment period. The fish were held in the saline solution for 120 minutes and were then released immediately into fresh water. Observation was made of the number of dead or inactive fish at 0, 10, and 30 minutes after release. A final count of dead fish was made 18 and 24 hours later when all survivors appeared to be fully recovered.

RESULTS

The combined results of these tests are shown in table 15. Fish from lots II and VIII suffered a little less mortality than the control, but these differences were not significant. The reaction of lot XI was almost identical to that in the control. However, the other treated lots lost considerably more fish.

TABLE 15.—*Distress and mortality among fish exposed to a 4-percent solution of NaCl for 2 hours*

	Number of fish	Average weight of fish (grams)	Percent of fish dead or inactive after release			Percent mortality after 24 hours	Average weight of dead fish (grams)
			At 0 minutes	At 10 minutes	At 30 minutes		
Lot I	80	205	58	42	49	30	190
Lot II	20	265	75	65	70	25	260
Lot III	20	275	100	95	90	70	280
Lot IV	40	230	92	80	80	53	240
Lot V	40	225	95	92	88	72	220
Lot VI	40	240	82	72	75	58	235
Lot VIII	14	260	57	43	43	21	165
Lot IX	60	215	98	92	92	82	215
Lot X	41	220	88	90	88	80	215
Lot XI	61	230	57	59	61	31	195

DISCUSSION

The results were inconclusive and data from these tests have been presented primarily for possible comparison with other work. The resistance shown by lots II and VIII may reflect superior physical condition of the few fish remaining in these lots. This does not explain a lower resistance in lot III which had previously displayed reactions almost identical to those of lot II. A relation between resistance to this form of stress and DDT treatment may be indicated by the higher-than-control mortality shown by six of the seven treated lots, excluding lots II and VIII.

PATHOLOGY

Examination of tissues from fish of all lots was made for evidence of pathology. Tissue changes due to parasitism were apparent throughout the experiment, but no pathology due to exposure to DDT was found.

Hematocrit measurements were routinely made on fish from all lots; no differences between treated and untreated fish were revealed. The hemoglobin decline reported in mammals was not reflected in hematocrit measurements on these trout exposed to DDT.

SUMMATION AND COMMENTS

The results of this study offer evidence of a threshold level of effectiveness of DDT in chronic poisoning of cutthroat trout. Low dosages of DDT, both in bath and in food, did not appear to affect death rate, growth, or reproductive success over an exposure period of about 20 months. The intermediate dosages given lots IV and IX had variable effects. Lot IV, given 0.1 p.p.m. DDT monthly in bath form, exhibited significantly higher mortality, similar size, and similar reproductive success when compared with the control group. Lot IX, treated once weekly with DDT in the diet at the rate of 0.3 milligrams per kilogram of body weight, did not differ from the control except, in a highly variable manner, residue buildup, and a nonsignificant increase in mortality during the last few months of the experiment. The higher dosages produced a statistically highly significant increased rate of mortality. This was the prime effect; the increase in size was a possible indirect effect based on high mortality of small, diseased, slow-growing fish. No direct effect on reproduction was observed in these lots, IV and IX, except an increased mortality of sac-fry which could not be quantitated; the reproductive potential of these fish was reduced both by this effect and by the high rates of mortality of the adult fish.

In part, at least, the threshold-type effect may be due to the limited ability of the fish to detoxify DDT. In almost every fish analyzed, three component chlorinated hydrocarbons were found: DDT, DDE, and DDD. The percentage of whole-body residues present as each of these components is presented in table 12. It can be seen that DDT ranged from about 90 percent in the higher fed groups to a low of 4 percent in the lowest fed group after one treatment. The lower-dosage-treated fish, although not different from controls in total residues, were higher in the relative amounts of DDT present and lower in DDE content. The basic diet, presumably the source of residues in the control fish, contained a chlorinated hydrocarbon mixture of about 60 percent DDE, 20 percent DDT, and 20 percent DDD. The residues in control fish were divided about 74 percent DDE, 16 percent DDT, and 9 percent DDD (average of all controls), indicating a higher rate of metabolism and/or excretion of the latter two components.

Enzymatic dehydrochlorination of DDT to DDE has been reported in insects (Perry and Hoskins, 1950). An enzyme capable of catalyzing this reaction in vitro has been isolated and purified (Lipke and Kearns, 1960). The enzyme does not act upon DDE, but will dehydrochlorinate DDD at approximately four times the initial rate of DDT. The in vitro formation of DDE from DDT has apparently not been reported using systems obtained from higher animals, but DDE has been found in human fat (Pearce et al., 1952) to the extent of 33 to 78 percent of the total chlorinated hydrocarbons. Mattson et al. (1953) reported it present in human fat to the extent of 39 to 86 percent and Hayes et al. (1958) to the extent of 33 to 90 percent of the total. The latter group also reported that smaller proportions of DDE were present in samples obtained from humans with the greatest exposure to DDT, a finding confirmed by the present work.

Little has been reported on the metabolic fate of DDT in higher animals. A water-soluble excretory product, 2,2-bis (p-chlorophenyl)acetic acid (DDA), has been found in the urine of DDT-treated rabbits (White and Sweeney, 1945), dogs (Ofner et al., 1945), and humans (Neal et al., 1946). Burns et al. (1957) found DDA or similar compounds to be the major excretory metabolite of DDT in the rat. Excretion was primarily through the bile and feces; urinary excretion assumed importance only when the bile duct was ligated. The presence of DDD in rat tissues as a metabolite of DDT, but not of DDE, has been reported by Peterson and Robison (in press). In our laboratory, DDD has been found inconsistently in fish tissues after field exposure to commercial preparations of DDT. The concentrations present in whole bodies of trout in this study probably do not represent accumulation by metabolic formation from DDT. There was a downward trend in the percentage of DDD present in these fish as the study progressed, suggesting that levels of DDT and DDE were building up at a faster rate than DDD. In another experiment (Bridges, Kallman, and Andrews, 1963), in which a warm-water pond was treated once with p,p′-DDT, we have found DDD present in fish and crayfish in increasing proportion since about 1 week after the treatment. After 14 months, DDD was present to the extent of 73 percent of the total chlorinated hydrocarbons in rainbow trout. Its identity was established by chromatography in two solvent systems, using known DDD as reference, and by passage through a column of MgO, producing a dehydrochlorinated derivative which was chromatographically identical to that of known DDD.

Analysis of tissue and organ pools obtained from fish which died during the experiment indicated a highly uneven distribution of total chlorinated hydrocarbons and of the three components. Distribution of the components seems not to be a function of the lipid content of the various organs. Even in those organs with high DDD contents, sufficient DDD was present in the basic diet to account for the total, assuming selective retention of of this substance by the organ. McKinley and Grice (1960) found that both DDD and DDT are retained in tissues to a greater extent than are similar substances. However, Finnegan et al. (1949), reported that in dogs DDD is both metabolized to DDA and excreted in the feces to a greater extent than DDT. As mentioned above, there is no evidence from our whole-body residue data that DDD was selectively retained in the control fish of the study.

No clear pattern of relative distribution emerges from the residue data of tissue pools. Brain, for example, was only slightly higher than ovary in lot II, but contained more than fivefold the residue of ovary in lot VIII. In the low-dosage pools, ovary contained higher residues than brain. Residues in muscle and liver were similar to each other in lots II and VIII, but with markedly different relative amounts of the three component chlorinated hydrocarbons. Muscle in all pools contained a high proportion of DDT, while liver was uniformly low in this component. Total residues in testes were low in three pools, but were the highest in the fourth.

Comparison of these results with the findings of Holden (1962) are of interest. Holden exposed brown trout to acutely lethal dosages of C^{14}-DDT in water. The calculated tissue concentrations, based on radioactivity and expressed as DDT, were generally higher in liver and muscle than in brain. The carbon tetrachloride-extractable material expressed as a percentage of the organ weight was higher in brain than in liver and muscle. A direct relation between the concentration of material extractable by this solvent and the DDT concentration in the organ did not exist after 16 hours of exposure. After 160 or 230 hours, such a relation did exist, except in brain. Brain was consistently high in "lipid" and low in DDT in this short-term study. At 16 hours, gill filaments were lowest in "lipid" and highest in DDT content. At 160 hours they were also lowest in DDT.

The question of the cause of death of fish with a given residue of a chlorinated hydrocarbon insecticide is often of importance from a management or forensic standpoint. Whole-body residues of total chlorinated hydrocarbons, however high, are insufficient data in most cases, especially when the symptoms exhibited by the fish either were not observed or were not typical of this type of poisoning. Often fish with extremely high residues appear normal; fish taken from Clear Lake, Calif., some time after an application of DDD had residues of over 2,000 p.p.m. in fat, but were in apparent good health (Hunt and Bischoff, 1960).

With the chlorinated hydrocarbons whose metabolic pathways are known in at least some detail, both the total residue and its distribution in terms of toxicant and metabolites should be of value in deciding upon the cause of death. In our work, fish treated with the highest levels of DDT exhibited early marked mortality and had smaller relative amounts of the metabolite DDE present. Although mortality was probably not caused directly by DDT, but rather by other stresses, it can be related to both total residue and extent of metabolism. Heaviest mortalities in these lots occurred before the maximum relative amounts of DDT were reached. This was perhaps a result of a population change; the fish which succumbed were not routinely analyzed for total body residues, but may have had higher relative DDT concentrations than survivors.

The high-dosage lots of this experiment may be considered as representing a state between acute and chronic exposure to DDT since heavy mortality was observed early in the study and occurred at a rapid rate. In the more truly chronic aspects, in the low-treatment groups (lots V, VI, X, and XI), there was an increased relative amount of DDT present, but neither mortality nor total residues differed significantly from lot I. The percentage of DDT present in fish of lots IV and IX gradually increased with increasing residues to a level intermediate to the high- and low-dosage lots. Mortality was high only in lot IV, and in this lot the increased mortality occurred both early in the study, when relative DDT content was higher than that of low-dosage lots, and later, when there was probably no significant difference in relative amounts of DDT and DDE between lots IV and V, but when total residues were higher in lot IV. In long-term exposures involving repeated dosages of DDT, mortality probably cannot be related to a "metabolic index" such as [DDE]/[DDT]. This may be due in part to a chronic effect of DDE, whose toxicity to fish has not as yet been determined.

Residue levels in individual organs may offer another important criterion in judging cause of death. The brain appears particularly promising in this respect, although further work must be done with acutely lethal doses of DDT. Brains of fish of lots II and VIII that died had total residues far higher than whole-body residues of living fish sampled at comparable times. This was not true of brains of fish which died spontaneously in lots V–VI and X–XI, in which mortality differed little from control levels.

At our present state of knowledge, the best means of assessing cause of death in fish exposed to DDT appears to be by judgment of the combination of factors: Total residue, metabolic index, and residue in the brain. No absolute "lethal levels" are known for any of these factors, and wide latitudes must be allowed in any given situation. Needless to say, a knowledge of the history of pesticide use prior to any fish kill is important.

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REVISED BIBLIOGRAPHY ON THE CUTTHROAT TROUT

March 3, 1849

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 65

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This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two Bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

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54. Fluctuations in Age Composition and Growth Rate of Cutthroat Trout in Yellowstone Lake, by Ross B. Bulkley. 1961. 31 pp.
55. Mortality Studies on Cutthroat Trout in Yellowstone Lake, by Orville P. Ball and Oliver B. Cope. 1961. 62 pp.
56. Limnology of Yellowstone Lake in Relation to the Cutthroat Trout, by Norman G. Benson. 1961. 33 pp.
57. Chemical Composition of Blood of Smallmouth Bass, by Eddie Wayne Shell. 1961. 36 pp.
58. Chronic Effects of Endrin on Bluntnose Minnows and Guppies, by Donald Irvin Mount. 1962. 38 pp.
59. Predicting Year-Class Abundance of Yellowstone Lake Cutthroat Trout, by Ross V. Bulkley and Norman G. Benson. 1962. 21 pp.
60. Limnological Organic Analyses by Quantitative Dichromate Oxidation, by John A. Maciolek. 1962. 61 pp.
61. Bioassay and Use of Pituitary Materials to Spawn Warm-Water Fishes, by Howard P. Clemens and Kermit E. Sneed. 1962. 30 pp.
62. Equilibrium Yield and Management of Cutthroat Trout in Yellowstone Lake, by Norman G. Benson and Ross V. Bulkley. 1963. 44 pp.
63. Sea-Water Systems for Experimental Aquariums; A Collection of Papers, edited by John R. Clark and Roberta Clark. 1963. 190 pp.
64. Some Chronic Effects of DDT on Cutthroat Trout, by Don Allison, Burton J. Kallman, Oliver B. Cope, and Charles Van Valin. 1963. 30 pp.
65. Revised Bibliography on the Cutthroat Trout, by Oliver B. Cope. 1964. 43 p.

REVISED BIBLIOGRAPHY ON THE CUTTHROAT TROUT

Oliver B. Cope

RESEARCH REPORT 65

UNITED STATES DEPARTMENT OF THE INTERIOR
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ABSTRACT

This paper is a compilation of 221 abstracts of publications on the biology, culture, distribution, and management of the cutthroat trout, *Salmo clarki* Richardson. The 1958 publication, "Annotated Bibliography on the Cutthroat Trout," contained 135 abstracts, which have been incorporated with recent ones to form the present report.

REVISED BIBLIOGRAPHY ON THE CUTTHROAT TROUT

By Oliver B. Cope, Fishery Research Biologist

Fish-Pesticide Research Laboratory, Denver, Colorado
Bureau of Sport Fisheries and Wildlife

"Annotated Bibliography on the Cutthroat Trout" (Cope, 1958) brought together a compilation of 135 abstracts covering publications on the biology, culture, management, and distribution of *Salmo clarki* Richardson. Since 1958, several more articles dealing with the cutthroat have appeared in the biological literature. The present collection combines the abstracts published in the 1958 paper with those that have appeared through the middle of 1963.

The growing importance of *Salmo clarki* as an animal for experiment is evident from the recent references. Many studies on disease, nutrition, and pesticide toxicity are based on effects on the cutthroat, partly because of the availability of this species in areas in which these kinds of research are done, and partly because of improved cultural practices which reflect an improved understanding of the biology of the cutthroat.

Advanced investigations in the population dynamics of cutthroat, especially at Yellowstone Lake, are the subject of several recent publications. This new knowledge places fishery science in a favorable position with regard to understanding the bionomics of *Salmo clarki.*

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Adami, J. G.

1908. On a giant-celled rhabdomyosarcoma from the trout. Montreal Medical Journal, 37(3) : 163–165.

A tumor is described from a position between the dorsal and adipose fins, off the middorsal line, on a cutthroat trout. The tumor is a giant-celled one, originating in voluntary muscle.

Bachman, Roger Werner.

1958. The ecology of four North Idaho trout streams with reference to the influence of forest road construction. Master of science thesis, University of Idaho. 97 pp.

In this study of the effects of logging on streams in Idaho, data were collected after logging for comparisons of prelogging information collected by Oien. Fish species remained as before the logging, with coastal cutthroat as the dominant form. Coefficients of condition were not lower than before logging. Length-weight relations for populations from four streams were very similar. Food habits were summarized. In general, logging did not appear to affect cutthroat populations.

Ball, Orville P.

1955. Some aspects of homing in cutthroat trout. Proceedings of the Utah Academy of Science, Arts, and Letters, 32 :75–80.

Very little previous work has been done to measure the homing instinct in adfluvial fish. The U.S. Fish and Wildlife Service studied homing in the cutthroat in Yellowstone Lake from 1949 to 1955. Immatures in streams were fin clipped, and spawners in streams were tagged with Petersen disks. Results from immatures were not conclusive, but only 6 out of 280 repeat spawners returned to streams other than those in which they were originally tagged.

Ball, Orville P., and Oliver B. Cope.

1961. Mortality studies on cutthroat trout in Yellowstone Lake. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report 55. 62 pp.

In a study of the Yellowstone cutthroat trout, *Salmo clarki lewisi*, by the U.S. Fish and Wildlife Service, effects of environment on mortality of eggs, immature fish, spawners, and postspawners were measured for various components of the population in Yellowstone Lake (Wyoming). Five methods of estimating mortality of adults on spawning runs are described, with counting and tagging as the principal procedures. Of the total number of eggs deposited in the gravel, 60 to 75 percent died before hatching, and 99.6 percent had died by the time the fingerlings entered Yellowstone Lake. Among adults on spawning runs, mortality was 48.1 percent. Sex ratios on spawning runs were uneven, averaging 1.47 females to 1 male. Very few trout spawned a second time; of those that did, more spawned after a 2-year interval than after 1 year.

In the Arnica Creek runs, 48.6 percent died in the stream, 40.2 percent died later in the lake of natural causes, 7.6 percent were taken by fishermen, and 3.6 percent were alive 2 years later. The white pelican is a serious predator on cutthroat trout in Yellowstone Lake. From 1949 to 1953, fishermen caught 11.6 percent of the catchable trout available to them. Migrations of adult fish in Yellowstone Lake were traced through tagging.

Bangham, Ralph V.

1951. Parasites of fish in the Upper Snake River drainage and in Yellowstone Lake, Wyo. Zoologica, 36, Part 3(16) : 213–217.

Parasites of 291 Yellowstone cutthroat are listed; 278 of the fish examined were infested. Sixteen species of parasites are named, and the incidences of several are noted in discussions.

Bangham, Ralph V., and James R. Adams.

1954. A survey of the parasites of freshwater fishes from the mainland of British Columbia. Journal of the Fisheries Research Board of Canada, 11(6) : 673–708.

In this study of parasites of British Columbia fishes, 178 out of 179 cutthroat from 11 locations were infested. Eighteen species of parasites were found, with trematodes, nematodes, cestodes, and acanthocephalans having the highest incidence.

Barrett, I.

1951. Fertility of salmonoid eggs and sperm after storage. Journal of the Fisheries Research Board of Canada, 8(3)125–133.

Effects of storage on fertility of salmonoid eggs have been studied. Mature eggs and sperm were stripped into dry sterile jars and stored for varying periods at low temperatures before testing their fertility with fresh genital products. Eggs of the chum salmon, *Oncorhynchus keta*, were stored at temperatures of 2.5° to 5.8° C. for 108 hours with an average infertility of less than 20 percent. The sperm of this species shows a wide variation in its reaction to storage, but may be retained for 36 hours in sterile jars with an average of less than 10 percent infertility. Cutthroat eggs stored 36 hours were 18 percent infertile. Kamloops and cutthroat confirmed the applicability of the technique of transporting eggs and sperm stored together in the absence of water.

Beal, Fred R.

1959. For the cutthroat, a brighter outlook. Wyoming Wildlife, 23(8) : 7–9.

Differences between Yellowstone and Snake River cutthroat are discussed. The use of cutthroat eggs of these stocks is described, and the results of planting Snake River cutthroat explained. Recent management trends in Wyoming and Idaho are discussed.

Bean, Tarleton H.

1894a. Life history of the salmon. Bulletin of the U.S. Fish Commission, 12 : 21–38.

The red-throated trout, *Salmo mykiss*, is called Clark's trout and Rocky Mountain trout. Discussion of distribution, food-habitat preferences, and habits is given.

1894b. Bibliography of the Salmonidae of Alaska and adjacent regions. Bulletin of the U.S. Fish Commission, 12 : 39–49.

Lists several very early papers pertaining to cutthroat trout.

Benson, Norman G.

1958. Hermaphroditism in the cutthroat trout. Copeia, (3) : 239–240.

A second record of hermaphroditism in the Yellowstone cutthroat was found in 1957. The arrangement of gonads was different from that in the first record. Here, a ripe testis was found posterior to the left ovary.

1960. Factors influencing production of immature cutthroat trout in Arnica Creek, Yellowstone Park. Transactions of the American Fisheries Society, 89(2) : 168–175.

Data are presented on the variations in production of immature cutthroat trout from Arnica Creek, a tributary of Yellowstone Lake, from 1951 to 1958. Of 36,786 immature trout counted, 87.3 percent were age group 0. The highest production occurred during years when large numbers of age group 0 trout migrated downstream to Yellowstone Lake from mid-July to mid-August, or soon after they emerged from the gravel. Ecological conditions associated with a large July–August migration were receding water levels and much light. High productions of age groups I and II trout, which are predaceous on fry, did not appear to have as much influence on the production of good year classes as did the downstream migration pattern. No relation was evident between year-class variation of immatures and reappearance of these same year classes in the spawning runs. The number of spawners or the time of the spawning run did not bear any consistent relation to the production of immatures.

1961. Limnology of Yellowstone Lake in relation to the cutthroat trout. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report 56. 33 p.

Limnological data collected from 1954 to 1959 on surface currents, temperatures, bottom soils, water chemistry, plankton, bottom fauna, and higher aquatic plants are related to the biology of the cutthroat trout, *Salmo clarki lewisi*, in Yellowstone Lake (Wyoming). The lake, formed on Eocene lava, is oligotrophic and low in dissolved solids. *Diaptomus shoshone, Daphnia shoedleri,* and *Conochilus unicornis* made up more than 90 percent of the macroscopic zooplankton by number. Upwelling in West Thumb was demonstrated by temperature stratification and conductivities. Plankton distribution and abundance are related to currents and water chemistry. Depth distribution, feeding, and movements of trout are related to food abundance. Trout less than 315 millimeters in total length are not caught readily because of their feeding habits and greater dispersal. There is evidence that the heavy trout harvest in the northern part of the lake allowed *Gammarus lacustris*, an important trout food, to become locally abundant.

Benson, Seth B., and Robert J. Behnke.

1961. *Salmo evermanni* a synonym of *Salmo clarkii henshawi.* California Fish and Game, 47 (3) : 257–259.

Specimens of *clarkii henshawi* from Lake Tahoe, Calif., were compared with type material of *evermanni.* It is concluded that the two are synonymous.

Biesinger, Kenneth E.

1961. Studies on the relationship of the redside shiner (*Richardsonius balteatus*) and the longnose sucker (*Catostomus catostomus*) to the cutthroat trout (*Salmo clarki*) population in Yellowstone Lake. Master of science thesis, Utah State University. 92 pp.

The biology of longnose suckers and redside shiners in Yellowstone Lake and tributaries was studied in relation to that of the cutthroat. Interactions among species occur in lagoons and other protected areas. Suckers and shiners were not found to damage trout redds or compete with trout for spawning space. There was an overlap of food habits of juveniles and adults of all species, suggesting competition in certain areas. This competition does not appear to be great, except in younger fish. Control of suckers and shiners is suggested for management, and items for further study are pointed out.

Bilton, T. H.

1952. The creel census of cutthroat trout at Lakelse Lake, 1952. Fisheries Research Board of Canada, Progress Reports of the Pacific Coast Stations, No. 92, October: 18–20.

Three years of creel-census data were accumulated for cutthroat in the lake and river fishery in the Lakelse drainage. Lake fishing was best in July, during mayfly hatches. The river fishery had moved to the lake by this time. In August and September most of the fishing was in the lake.

The fishery was probably exploiting less than 10 percent of the catchable stock annually. A heavier fishery would obtain a higher yield, probably over a long period of years.

Bilton, T. H., and M. P. Shepard.

1955. The sports fishery for cutthroat trout at Lakelse Lake, British Columbia. Fisheries Research Board of Canada, Progress Reports of the Pacific Coast Stations, No. 104, November: 38–42.

Since 1950 the Fisheries Research Board of Canada has kept records on cutthroat in Lakelse Lake and Lakelse River. Notes on feeding and spawning are given. The sport fishery is described. About 2,000 cutthroat are taken annually by this fishery. Age III and IV trout have predominated in the catch each year.

Figures on the catch per unit of effort indicate that no severe depletion has occurred in the lake population, but catches on the river have declined since 1950.

Anglers have removed an average of 7 percent of the lake population annually, and 18 to 31 percent of the river population.

Bjornn, Ted C.

1957. A survey of the fishery resources of Priest and Upper Priest Lakes and their tributaries, Idaho. Idaho Fish and Game Department, Completion Report. 176 pp.

In 1955 a survey of the fishery resources of Priest and Upper Priest Lakes and their tributaries was initiated to determine the factors causing the decline in the number of cutthroat in the lake.

Streams running into the lakes provided adequate spawning sites for both cutthroat and Dolly Varden trout. In streams where brook trout have become abundant, competition for food and space between brook trout and cutthroat is very intense. Competition is less in the lakes.

Of the 93,300 fish caught in 1955 from these lakes, 10 percent were cutthroat, and of the estimated total catch of 111,400 in 1956, 5 percent were cutthroat. The average rate of catch for cutthroat in the upper lake was 1.02 fish per boat per hour, compared with 0.70 in Priest Lake. The average-size cutthroat caught in 1956 was between 11.1 and 11.2 inches. The body-scale relation appeared to be approximately linear.

Juvenile cutthroat spawned from fish migrating from the lakes were found to spend an extended period of time in the streams before migrating to the lakes. Of the fish collected from Priest Lake in 1956, 38 percent spent 2 years in the streams before migrating, 57 percent 3 years, and 5 percent 4 years. For the upper lake, 6 percent migrated after their first year, 35 percent after their second, and 58 percent after their third. The spawning season begins in April and generally ends July 1. Cutthroat in the Priest Lakes mature predominantly during their fifth year of life. Only a very small number of fish ever spawn a second time.

Diet of the cutthroat consists mainly of insects and some plankton.

An average of nearly 500,000 cutthroat a year, mostly fry, have been planted in the Priest Lake drainage for at least 10 years, but less than 1 percent have returned to the creel.

1958. The Priest Lakes fishery. Idaho Wildlife Review, 11(1) : 3–6.

The Priest Lakes are described with their fish fauna. The cutthroat fishery has declined. The life history of the cutthroat here is reviewed. Most spawners are 5 years old. The decline of the cutthroat is attributed to overfishing and competition of immatures with other species in streams. Fishing times and places are described.

1961. Harvest, age structure, and growth of game fish populations from Priest and Upper Priest Lakes. Transactions of the American Fisheries Society, 90(1) : 27–31

Priest and Upper Priest Lakes in norther Idaho produced a catch of approximatel 100,000 fish in 1956. The catch was compose of kokanee (93 percent), cutthroat trout (percent), and Dolly Varden and lake trout (percent). Three out of four fishermen wer nonresidents, and a third of the hours fishe were expended by anglers from more than 60 private cabins. The average rate of catch fo kokanee was 2.5 fish per boat per hour on Pries Lake and 0.9 on Upper Priest Lake; for cutthroat trout it was 1.0 fish per boat per hour o the upper lake and 0.7 on Priest Lake. Juvenile cutthroat trout were found to spend 2 t 3 years in tributary streams before enterin the lakes. The catch and spawning escapements of kokanee from Priest Lake during 195 and 1956 were composed chiefly of fish in thei fifth year of life.

Black, Edgar C., and Isadore Barrett.

1957. Increase in levels of lactic acid in the blood of cutthroat and steelhead trou following handling and live transportation. Canadian Fish Culturist, Issue 20 pp. 13–23.

Alterations in the blood levels of lactic aci were investigated in hatchery-raised coasta cutthroat and steelhead trout in the following conditions: (a) After transferring from outside ponds to troughs inside the hatchery 6 yards (54.9 meters) away; (b) sham transportation run for 2 hours in tank truck; (c) holding cutthroat for 40 hours, steelhead fo 16 hours, in hatchery troughs before transportation of 2 hours 50 minutes.

The following results were noted: (1) Significant increases in blood lactic acid in bot species as a result of minimal handling, i.e., i transporting the fish from outside ponds t inside hatchery troughs; (2) very significan increase in blood levels of lactic acid followin sham transportation run for 2 hours in a tan truck, and levels were higher in both specie than those observed for lake trout and Kamloops trout in 1955; (3) storage of cutthroa for 40 hours resulted in significant lowerin of blood level of lactic acid (range 0.8–17.5 mg percent) following transportation run of hours 50 minutes. Storage of steelhead trou for 16 hours before the same transportatio run of 2 hours 50 minutes resulted also in lower blood level of lactic acid (range 9.5–88 mg. percent).

Bonham, Kelshaw, and R. Walter Williams.

1948. Effect of population pressure upon rate of growth and food conversion of fingerling cutthroat trout. Progressive Fish-Culturist, 10(1) : 15–18.

Fingerling cutthroat trout in 80-gallon aquariums were fed equal amounts of food per fish; 2 tanks had 28 fish each, 2 had 280 fish each. Lengths and weights were determined on four occasions. Crowded fish did not differ significantly from uncrowded ones in growth rate or food-conversion rate.

Brown, C. J. D., and Charles Buck, Jr.

1939. When do trout and grayling fry begin to take food? Journal of Wildlife Management, 3(2) : 134–140.

Experiments on time of initial feeding were done for grayling and several trouts, including cutthroat. Some cutthroat fry were feeding 14 days after hatching, and all were feeding 23 days after hatching. Little uniformity between species was shown; rainbow began first, brook trout last. Size or presence of yolk sac showed no correlation with initial feeding time.

Brown, C. J. D., and Jack E. Bailey.

1952. Time and pattern of scale formation in Yellowstone cutthroat trout, *Salmo clarkii lewisi*. Transactions of the American Microscopical Society, 71(2) : 120–124.

Scales from adult and immature Lake Yellowstone cutthroat were studied, and three kinds of scale development noted: (1) Fish completely scaled before their first winter and with annuli on all scales; (2) fish partially scaled before the first winter, some scales without annuli; (3) fish without scales in their first winter, and with no annuli at the end of the first growing season.

Bulkley, Ross V.

1959. Report on 1958 fishing studies by the Bureau of Sport Fisheries and Wildlife on Rocky Mountain National Park. Rocky Mountain Sport Fisheries investigations, administrative report (mimeographed). 38 pp.

In a creel census and shocking program in waters of Rocky Mountain National Park, catch and population data were collected. Cutthroat trout were important in the catch. Growth data were calculated from scale readings, and cutthroat growth was found to be slower than other trout growth in the same waters. Surveys of high mountain lakes and streams revealed the distribution of cutthroat in the park, and gave clues to the fates of some planted fish. Stomach contents of cutthroat from some lakes and streams were tabulated and are presented in tables.

The green-back trout, *Salmo clarki stomias*, was studied in the Upper Big Thompson River. Information on length, growth, age, migration, food habits, reproduction, and sex ratios was obtained from fish captured in an upstream-downstream trap. Preliminary work was done to create a sanctuary for this rare cutthroat.

1961. Fluctuations in age composition and growth rate of cutthroat trout in Yellowstone Lake. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report 54. 31 pp.

Age composition, growth rate, and year-class strength of Yellowstone Lake cutthroat trout from collections made in 1948 and from 1950 to 1959 are analyzed to relate total catch to changes in age composition and growth rate. Catch increased from 200,015 cutthroat in 1950 to 393,467 in 1959. An increase in growth rate of fish fully recruited to the fishery and a decrease in percentages of fish belonging to age groups VI and VII are attributed to an increase in fishing pressure. Mean age of the catch varied with year-class strength, but was essentially unaffected by changes in size of the catch. Mean length of the catch has remained high, suggesting that production is more efficient now than in past years. Maximum equilibrium yield may be near.

If the catch continues to increase at the present rate, it may become excessive within the next few years.

1963. Natural variation in spotting, hyoid teeth counts, and coloration of Yellowstone cutthroat trout. U.S. Fish and Wildlife Service, Special Scientific Report—Fisheries No. 460. 11 pp.

Differences in hyoid teeth and spotting counts among samples from four related populations of Yellowstone cutthroat trout, *Salmo clarki lewisi* Girard, reached species and subspecies levels as defined by some workers. Body coloration varied significantly among fish in six spawning runs of Yellowstone Lake, Wyo. The use of coloration, spotting, and hyoid teeth counts in cutthroat trout for taxonomic purposes needs reevaluation.

Bulkley, Ross V., and Norman G. Benson.

1962. Predicting year-class abundance of Yellowstone Lake cutthroat trout. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report 59. 25 pp.

Fluctuations in strength of year classes from 1946 to 1956 of Yellowstone Lake cutthroat from Pelican and Chipmunk Creeks are compared with the parental stock and several climatically influenced factors of the environment. Variations in year-class strength in the two tributaries were highly correlated with fluctuations in lake water levels. Strong year-classes occurred in years of low water. Female spawner escapement, timing of the runs, and summer air temperatures were not significant factors.

A formula based on water levels is presented for predicting year-class strength in Pelican Creek and in the Fishing Bridge area fishery.

Stocking of fry in years of high water is suggested as a means of supplemental natural production. A method of forecasting lake water levels several months in advance of their occurrence is discussed.

CALHOUN, A. J.

1944a. Black-spotted trout in Blue Lake, California. California Fish and Game, 30(1) : 22–42.

Upper Blue Lake is an oligotrophic lake at 8,130-foot elevation in the California Sierra Nevada. The lake has black-spotted trout which spawn in five tributary streams. Spawning time, size of spawners, and age of spawners are discussed.

In 1941 a significant number of the trout which matured sexually failed to ascend a stream to spawn.

Marked trout showed that growth did not take place after spawning, and few spawned a second time.

Heavy annual plants of small fingerlings, coupled with closure to fishing, over a 6-year period, did not increase the number of fish in the spawning runs. It seemed to lead to an overpopulation of fish and a depletion of food.

It appears undesirable to return spent spawners to a lake like Blue Lake.

1944b. The food of the black-spotted trout (*Salmo clarkii henshawi*) in two Sierra Nevada lakes. California Fish and Game, 30(2) : 80–85.

The black-spotted trout in Upper Blue Lake, a deep granite basin at 8,130-foot elevation, subsisted largely on chironomid larvae and pupae. They failed to utilize the rather abundant population of the minnow, *Rhinichthys oscula*, which competes with young trout for food.

The trout of Heenan Lake, a former grazing meadow at 7,000 feet transformed by a dam into a 25-foot-deep reservior, subsisted largely on plankton crustacea (daphnia and copepods). The extremely abundant bottom fauna of the littoral zone provided only 17 percent of their food (scuds of the *Gammarus type*) ; immature chironomids, plentiful in bottom samples, provided less than 9 percent.

Heenan Lake trout were in much better condition than those of Blue Lake, reflecting the relative richness of the two lakes.

CALIFORNIA DEPARTMENT OF FISH AND GAME.

1956. Cutthroat nursery. Outdoor California, 17(6) : 6–7, 11.

Heenan Lake, on the east slope of the Sierra Nevada in California, was built in the late twenties and stocked with cutthroat (*Salmo clarkii henshawi*) from Alpine County. The fish grew well and egg taking was initiated. California and Nevada have taken 19 million cutthroat eggs froh Heenan Lake for stocking elsewhere. California is attempting to reestablish the cutthroat in its former waters on the east slope.

CARLANDER, KENNETH D.

1950. Handbook of freshwater fishery biology. Wm. C. Brown Co., Dubuque, Iowa. 281 pp.

Contains life history and biological measurements reported in many papers on cutthroat.

1953. Handbook of freshwater fishery biology with the first supplement. Wm. C. Brown Co., Dubuque, Iowa. 429 pp.

Contains life history and biological measurements reported in many papers on cutthroat.

CHAPMAN, WILBERT MCL., and ELMER QUISTORFF.

1938. The food of certain fishes of North Central Columbia River drainage, in particular, young chinook salmon and steelhead trout. Washington Department of Fisheries, Biol. Report No. 37A. 14 pp.

In this study of food eaten by several species of fishes and of efficiency of foods in producing fish, 23 cutthroat stomachs were examined. Details of diet composition are presented. The cutthroat diet was similar to that of steelhead, with insects dominating the diet.

CODE, MARK R.

1950. Cutthroat trout without dorsal fins. Progressive Fish-Culturist, 12(2) : 85–86.

Cutthroat without dorsal fins were found in South Fork of Sheep Creek in the Ashley National Forest in Utah in 1936. The coloring of the abnormal fish was darker than that of normal cutthroat in the same stream. Some fish with deformed dorsal fins were found in the same stream.

Cope, E. D., and H. C. Yarrow.

1875. Report upon the collections of fishes made in portions of Nevada, Utah, California, New Mexico, and Arizona during the years 1871, 1872, 1873, and 1874. Zoology of the Wheeler Survey, 5 : 637–700.

Life history account of *Salmo virginalis*, called lake trout, brook trout, and speckled trout, with account of spawning, feeding, and movements. Angling methods are discussed. A discussion of Rio Grande trout, *Salmo spilurus* and *Salmo pleuriticus*, is presented.

Cope, Oliver B.

1953a. Chloretone as an anaesthetic for adult cutthroat trout. Progressive Fish-Culturist, 15 (1) : 35.

Chloretone was sucessfully used for anaesthetizing adult cutthroat at Yellowstone Lake in 1952. Stock solution was prepared in the laboratory, diluted in the field, and used on cutthroat at a strength of 1 : 2,000. Cost of the chloretone is slightly higher than that of urethane.

1953b. Length measurements of Lake Yellowstone trout. U.S. Fish and Wildlife Service, Special Scientific Report—Fisheries No. 103. 17 pp.

Conversion factors and tables are given for total length, fork length, and standard length of cutthroat from Yellowstone Lake. Comparisons from year to year of average total lengths from the fishery and from spawning runs show that declines in size took place from 1950 to 1952 in the northern part of the lake, where fishing was heaviest.

1955. The future of the cutthroat in Utah. Proceedings of the Utah Academy of Science, Arts, and Letters, 32 : 89–93.

The original cutthroats in Utah, the Utah cutthroat and the Colorado cutthroat, have been replaced by the Yellowstone cutthroat. Three factors threaten the cutthroat in Utah and in other Rocky Mountain States. Construction of dams has blocked spawning runs, and more dams are planned. Rainbow are being planted in cutthroat waters and replacing the cutthroat. Importation of cutthroat eggs from Yellowstone Park has been stopped, eliminating about 45 percent of the supply to Utah.

1956. Some migration patterns in cutthroat trout. Proceedings of the Utah Academy of Science, Arts, and Letters, 33 : 113–118.

Migrating cutthroat trout spawners in Arnica Creek, Yellowstone National Park, were counted, day and night, in 1951. Five-sixths of the total count for the season entered the stream between the hours of 6 p.m. and 7–8 a.m. This nocturnal migration is unusual for salmonid spawners, and is explained by the response of spawners to evening freshets that occur during the season of melting snows.

Lakeward-migrating fry in the same stream were counted, and it was found that 88 percent of the total count from August 1 to 15 moved downstream at night. Nocturnal movements of fry have been observed in other trouts and in salmon, and have been attributed to a negative phototropism.

1957a. Six years of catch statistics on Yellowstone Lake. Transactions of the American Fisheries Society, 85 : 160–179.

Catch statistics on cutthroat in Yellowstone Lake are reported for the years 1950–55. This fishery has only one species, the Yellowstone cutthroat. Hourly distribution of fishing is unusual in that there is practically no fishing before 7 a.m. and there are three peak periods of fishing throughout the day. There is an unusual uniformity of fishing effort among days of the week. Characteristics of the various components of the fishery are described.

Reduction of the daily catch limit from five to three fish in 1953 did little toward reducing total catch, but a shortened season had a decided effect on reduction of catch.

1957b. Races of cutthroat trout in Yellowstone Lake. U.S. Fish and Wildlife Service, Special Scientific Report—Fisheries No. 208, pp. 74–84.

Yellowstone cutthroat in tributaries to Yellowstone Lake and in Yellowstone River were studied to determine the existence of races. Tagging showed the association of groups of fish with particular spawning streams, and suggests that each tributary studied has its own race of cutthroat. Migrational patterns in the lake after spawning are quite constant from year to year. Mixing of races takes place in the lake, and each part of the lake appears to contain about the same mixture each year.

Times of migration into five streams were examined for 5 years, and five different patterns were perceived. The patterns were very constant from year to year.

Differences in mean total length of fish in spawning runs were measured, and significant differences found between streams. The differences may be due to differences in age composition or in growth rate, but are racial, since the same relations occur year after year.

Sizes of eggs and numbers of eggs per female were compared in relation to sizes of fish. Counts and measurements do not suggest that the fish are all of the same race.

1957c. The choice of spawning sites by cutthroat trout. Proceedings of the Utah Academy of Science, Arts, and Letters, 34 : 73–79.

Several environmental factors were examined in stream surveys on six Yellowstone Lake tributaries used by cutthroat trout for spawning. Attempt was made to relate distribution of redds in each stream with factors known to sometimes influence choice of spawning sites. Distribution of the best gravel and presence of waterfalls, log jams, and chemical barriers can account for distribution of nests in most of the streams. No explanation is at hand, with our limited knowledge, to reveal why more spawning did not take place in upstream areas in Chipmunk Creek; spawners shunned what seemed to be ideal spawning territory in middle stretches and used lower sections.

Influences of forest cover and stream gradient on distribution of spawning in streams were studied, but no effects were apparent.

A theory applying to use by migrating spawners of headwater areas by the first part of the migration and downstream sections by the later parts of the run was examined in the light of experience with cutthroat in these streams. Arnica, Chipmunk, and probably Pelican Creek fish support the theory; Clear Creek fish were the only ones appearing to refute the hypothesis.

1958a. Annotated bibliography on the cutthroat trout. Fishery Bulletin of the U.S. Fish and Wildlife Service, 58(140) : 417–442.

Publication of articles on the cutthroat trout began before the turn of the century, after exploration in the intermountain region had brought several forms of the cutthroat trout to the attention of fishery workers. The present compilation brings together 135 abstracts of papers which deal with the biology, culture, distribution, and management of the cutthroat. Twenty-seven zoological names proposed for the cutthroat are listed, and 70 vernacular names, taken from the literature, are presented.

1958b. Incidence of external parasites on cutthroat trout in Yellowstone Lake. Proceedings of the Utah Academy of Science, Arts, and Letters, 35 : 95–100.

Fish and Wildlife Service biologists determined some features of the external parasites on *Salmo clarki lewisi* in Yellowstone Lake. They counted and tabulated numbers of copepods (*Salmincola* sp.) and leeches (*Piscicola salmositica*) on 10,700 cutthroat trout in the 1956 and 1957 spawning migrations out of Yellowstone Lake. Comparisons of incidence of copepods, using analysis of variance and *t* tests, showed that statistical differences in degree of parasitism in 1957 did occur, and the Arnica Creek fish were more heavily infested than fish from other streams. With respect to the leeches, Arnica Creek fish also showed significantly greater numbers than those in four other tributary streams.

Arnica Creek fish showed no statistical difference in numbers of copepods or leeches between 1956 and 1957. Comparisons of parasitism on host sexes showed that male cutthroat bore more copepods then did females, but female trout had more leeches than did males.

Trout on the upstream migration in Arnica Creek bore more copepods and leeches than did the same fish moving downstream after spawning. It is supposed that many parasites were rubbed off the hosts during spawning.

Copepods in all streams were most abundant at the beginnings and ends of the trout migrations; the smallest numbers were found in the middle of the season. In three streams, greatest leech incidence was noted at the beginnings of the migrations with no leeches after the middle of the runs; one stream had the greatest leech density on its fish at the end of the season.

1961. Effects of DDT spraying for spruce budworm on fish in the Yellowstone River system. Transactions of the American Fisheries Society, 90(3) : 239–251.

DDT was sprayed at 1 pound per acre from airplanes for the control of spruce budworm on 71,678 acres in the Yellowstone River drainage in 1957. Stream-bottom invertebrates were reduced in number immediately after the spray. Recovery to near-normal total numbers occurred within a year in most streams, but species composition was altered. Drift samples in one stream showed dead and dying invertebrates to be drifting in the current in great numbers. No mortality to fish was found. Chemical analysis showed that DDT up to 0.03 p.p.m. was in several samples of water from streams. In one case a trace was found 55 miles downstream from the spray area. Vegetation samples contained up to 2.3 p.p.m. of DDT. All of the 80 samples of mountain whitefish (*Prosopium williamsoni*), cutthroat, rainbow, and brown trout (*Salmo clarki, Salmo gairdneri, and Salmo trutta*) analyzed contained either DDT up to 14.0

p.p.m. or DDE up to 6.54 p.p.m., or both. DDT was found in trout 85 miles below the spray area, and fish taken more than 2 years after spraying contained DDT.

Cordone, Almo J., and Don W. Kelley.

1961. The influences of inorganic sediment on the aquatic life of streams. California Fish and Game, 47(2) : 189–228.

This is a review of investigations on the effects of inorganic sediment on the aquatic life of streams. The direct effect of sediment upon fishes, the influences of sediment upon eggs and alevins, bottom organisms, aquatic plants, chemical and physical characteristics, and fish habitat and fish populations are discussed. Studies on cutthroat trout are reviewed and discussed in the light of effects of sediment It is concluded that sediment is detrimental to aquatic life in salmon and trout streams.

Cramer, Frederick K.

1940. Notes on the natural spawning of cutthroat trout (*Salmo clarkii clarkii*) in Oregon. Proceedings of the Sixth Pacific Science Congress of the Pacific Science Association, 3 : 335–339.

This study at Alsea Hatchery in Oregon was concerned primarily with spawning activities of coastal cutthroat. Time of spawning: spawning migrations were counted from December 5, 1938, to February 17, 1939, indicating a peak in late November and early December. Time of hatching: eggs hatch in 45–50 days. Nature of gravels and nests: eggs were 4 to 7 inches deep in pea-size gravel. Spawning activity: spawning day and night. Spawning was completed in 2 days. More than one nest per female was built. Eggs removed from nest: proportion of fertile live eggs 90 percent and better at stages up to 17 days. Egg counts: 2-year-old hatchery trout averaged 372.5 eggs per female. Small, wild, resident cutthroat averaged 480, and large, sea-run cutthroat averaged 1,170 eggs per female. Sex ratio: 52 males to 48 females in the spawning run.

Daniels, T. W.

952. Two-Ocean Pass, Wyoming. Wyoming Wildlife, 16(6) : 4–8.

Two-Ocean Pass, on the Continental Divide in Wyoming, is described geographically, and historical significance of fishing studies there is discussed. This was the route used by cutthroat to enter Yellowstone Lake from the Snake River, according to the author, who cites Evermann.

Davis, H. S.

1947. Care and diseases of trout. U.S. Fish and Wildlife Service, Research Report 12, Third Revision. 98 pp.

Care of trout at the hatchery, including care of ponds and raceways, is treated at some length. This is followed by general discussion of trout foods and methods of feeding, special attention being paid to use of dry products for supplementing fresh meat in the diet. Some consideration is given improvement of brood stock and its practical value.

General discussion of parasites and diseases of trout and their control is followed by detailed account of each disease, including characteristic symptoms, etiology, pathology, and methods of control. Figures include drawings and photomicrographs of the most important organisms that cause trout diseases and their effects on tissues.

Numerous examples of care and diseases of cutthroat trout are used in this report.

1953. Culture and diseases of game fish. University of California Press, Berkeley and Los Angeles. *x*+332 pp.

Cultural methods applicable to cutthroat and other trouts are discussed, and diseases and parasites, some of which occur in cutthroat, are taken up in detail.

DeWitt, John W., Jr.

1954a. A survey of the coast cutthroat trout, *Salmo clarki clarki* Richardson, in California. California Fish and Game 40(3) : 329–335.

Distribution of coastal cutthroat in California, morphology, life-history notes, and age distribution are discussed.

1954b. A survey of private trout enterprises in the west. Progressive Fish-Culturist, 16(4) : 147–152.

This paper deals with types of trout businesses, status of ownership, length of time in business, investment and income, egg and trout production, and important general problems in private trout production. Cutthroat trout production in Western States is listed in tables.

Dimick, R. E., and Don C. Mote.

1934. A preliminary survey of the food of Oregon trout. Oregon State College, Agricultural Experiment Station, Bulletin 323. 23 pp.

Insects are the dominant food of cutthroat and rainbow trout in Oregon. Aquatic insects are the bulk of the food. Terrestrial insects are prominent in diet in spring, summer, and fall. Crayfish, small fish, and pieces of dead salmon are important to large trout, while immatures of blackflies and midges are important to small trout in streams. Small trout in lakes eat mainly immature midges and water fleas. Fresh-water shrimps are important to Oregon trout in lakes. Table II has detailed analysis of food of 326 cutthroat trout.

DIMICK, R. E., and FRED MERRYFIELD.

1945. The fishes of the Willamette River system in relation to pollution. Oregon State College, Engineering Experiment Station, Bulletin 20. 58 pp.

Details of distribution of coastal cutthroat in this drainage are given. Two biological phases of the cutthroat are reported for the Willamette system. One is nonmigratory and does not get down to the main stem. The other is migratory and gets at least down to the main stem, and perhaps to the ocean. Migratory fish are larger, growing to 18–20 inches. Spawning and feeding habits are discussed. Relations to pollution are treated.

DONALDSON, LAUREN R., and FRED J. FOSTER.

1938. A summary table of some experimental tests in feeding young salmon and trout. Transactions of the American Fisheries Society, 67 : 262–270.

Tables contain many references to diets fed to cutthroat, and effects on the fish.

DONALDSON, LAUREN R., DONALD D. HANSLER, and TERRY N. BUCKRIDGE.

1957. Interracial hybridization of cutthroat trout, *Salmo clarkii*, and its use in fisheries management. Transactions of the American Fisheries Society, 86 : 350–360.

Two races of coastal cutthroat trout (*Salmo clarkii*) were hybridized, and resultant first generation hybrids were compared with parent races. In both laboratory and field studies the hybrids showed evidence of hybrid vigor. Catch of sport fishermen, during two seasons at Echo Lake in Washington, where both parent and hybrid trout were planted, was composed of 3 to 6 times as many fish of hybrid stocks as of parent races.

DRUMMOND, ROBERT A., and HOWARD A. TANNER.

1960. Reproduction and harvest of cutthroat trout at Trappers Lake. Colorado Cooperative Fishery Research Unit, 7 : 1–19.

Yellowstone and Colorado River cutthroat were studied in Trappers Lake. Numbers of spawners were determined, and Colorado River trout were 3.5 times as abundant as Yellowstone cutthroat. Production of fry was estimated. A total of 25,780 hours of fishing removed 11,601 trout from the lake.

DYMOND, J. R.

1928. The trout of British Columbia. Transactions of the American Fisheries Society, 58 : 71–77.

Description of cutthroat is given, with notes on distribution. Suggestions for management are advanced.

1931. Description of two new forms of British Columbia trout. Contributions to Canadian Biology and Fisheries N. S., 6(16) : 391–395.

Kamloops and cutthroat trout found at high altitudes in British Columbia differ in so many respects from those occurring at lower levels that it has been found advisable to recognize them as subspecifically distinct. Two new subspecies are therefore described, viz., *Salmo kamloops whitehousei* and *Salmo clarkii alpestris*.

1932. The trout and other game fishes of British Columbia. Canada Department of Fisheries, Ottawa, 51 pp.

Structural and color details of cutthroat in British Columbia are described. *Salmo clarkii clarkii*, *S. c. lewisi*, and *S. c. alpestris* are subspecies treated, and a hybrid between Kamloops and cutthroat is briefly discussed. Excellent color plates of coastal and Yellowstone cutthroats are included. Descriptions and life histories include colorations, morphologies, migrations, spawning, and game-fish qualities. Comparisons with steelhead are made for the coastal form.

ECHO, JOHN B.

1956. Some ecological relationships between yellow perch and cutthroat trout in Thompson Lake, Mont. Transactions of the American Fisheries Society, 84 : 239–248.

Relation between yellow perch and cutthroat trout in Thompson Lake in Montana was investigated in summers of 1952 and 1953. These lakes originally contained cutthroat trout and were later planted with yellow perch. Spawning time of yellow perch was in early May, of cutthroat trout in late May. The population of yellow perch was large, and growth was very slow. Although number of cutthroat trout was small, growth rate for this species was about average. Food of yellow perch was largely immature aquatic insects and plankton, while that of cutthroat trout was mostly mature aquatic insects and small perch. Yellow perch were commonly distributed along lake margins, and concentrations of perch fry and adults were found in these areas in spring. During this period the salmonid fishes were predominantly in deep water. Spot poisoning of yellow perch concentrations practically destroyed all fish in the treated area. Management suggestions are given.

Ellis, Max.

1914. Fish of Colorado. University of Colorado Studies, 11(1) : 1–136.

General discussion of trouts in Colorado; specific treatments of distributions and appearances of *Salmo c. spilurus*, *S. c. pleuriticus*, *S. c. stomias*, and *S. c. macdonaldi*.

Evermann, Barton W.

1893. A reconnaissance of the streams and lakes of western Montana and northwestern Wyoming. Bulletin of the U.S. Fish Commission, 11 : 3–60.

Deals with natural-history features of trout streams and distribution of fishes, as well as requirements for fish-cultural stations. Distributional and descriptive notes on *S. mykiss* are given.

Evermann, Barton W., and E. L. Goldsborough.

1907. The fishes of Alaska. Bulletin of the U.S. Bureau of Fisheries, 26 : 219–360.

Discussion of distribution of cutthroat trout in Alaska, description of the fish and comparison with rainbow, and fishing methods. Excellent colored plate.

Fish, Frederic F.

1934. Ulcer disease of trout. Transactions of the American Fisheries Society, 64 : 252–258.

Lesions of a type like furunculosis were noted on fingerling cutthroat, brook, rainbow, and lake trout and were caused by *Proteus hydrophilus*. Gross and microscopic pathology are described, and control is discussed. Photograph of lesions on cutthroat.

Fleener, G. G.

1952. Life history of the cutthroat trout, *Salmo clarki* Richardson, in Logan River, Utah. Transactions of the American Fisheries Society, 81 : 235–248.

This study of cutthroat trout in Logan River, Utah, was based on 306 specimens collected during 1948 and 1949 with an alternating-current electric shocking machine. Cutthroat trout comprised 24 percent of the anglers' catch in 1948 and 31.5 percent in 1949. The greatest numbers were found in areas with a high stream gradient. An estimated minimum number of 43.6 pounds of cutthroat per acre and 565 fish per mile was obtained from a series of shocking operations in 1949. Scale analysis was employed in determining age of the fish. With a scale magnification of 50 X, the body-scale (L/S) relation of cutthroat trout can be described as a curvilinear regression line, and expressed by the equation $L = 3.411S^{1.2006}$.

Smallest average annual growth increment was 53 millimeters. This was based on a growing season of approximately 200 days. Growth began in latter part of March and extended to early October.

Relation between standard length and weight was expressed mathematically as $W = 4.344 \times 10^{-5} L^{2.8253}$ where W equals weight in grams and L equals standard length in millimeters. Coefficient of condition decreased as length of fish increased. Male cutthroat trout had slightly more weight per unit of length than females. Of 142 fish marked with jaw tags, 15 were recovered; 9 moved out of the 0.1-mile area where they were released. Spawning period of cutthroat trout in this area lasted from early April to middle of August, but most fish spawned before middle of July. Adult females ranged in size from 11 inches downward. Slightly more females were present in a total of 35 separate collections.

Insects were the main item in diet of Logan River cutthroat trout. Ephemeroptera, Trichoptera, and Diptera were principal aquatic orders taken, while most important terrestrial order was Coleoptera. Small numbers of fish eggs eaten indicated that survival was not affected. Only two occurrences of fish were found in the entire food habits study. Small size of cutthroat trout in this area is in part attributable to almost total absence of forage fish in upper Logan River.

Foster, Fred J.

1933. Life histories and environmental needs of native and introduced fish. In "Game Management Developments and Needs," Utah State Agricultural College, Miscellaneous Publication No. 10. pp. 5–9.

Relations between cutthroat in Utah and introduced yellow perch are discussed. Kinds of cutthroat present in Utah, spawning conditions, characteristics of eggs and hatching habits of fry are presented. Causes of mortality are listed as nonfertilization, high water, and natural enemies. Life history of yellow perch is briefly discussed. Environmental needs of cutthroat are noted—character of water, food, and proper spawning conditions.

Gard, Richard.

1953. A taxonomic study of the rainbow trout populations of the streams on Mount St. Gorgonio, San Bernardino County, Calif.

Master of arts thesis, University of California. 59 pp.

This study was undertaken to determine if *Salmo evermanni* still existed in pure form. Several morphological features are correlated with type of environment. Given are: description of collecting areas, status of populations, sampling methods, comparisons of characteristics, effects of stocking. It is concluded that *S. evermanni* is not present in the area, and this is attributed to stocking with rainbow.

GEE, MERLE A.

1940. Report on the Upper Pecos River creel census, Santa Fe National Forest. Transactions of the Fifth North American Wildlife Conference. pp. 207–217.

A creel census was conducted in 1939 in the Upper Pecos River. Brown, rainbow, and Yellowstone cutthroat trout were the species caught. Brown trout, naturally reproduced, were the most abundant in the creel. Rainbows, stocked and naturally reproduced, were next in numbers in the creel. Cutthroat, planted and naturally reproduced, comprised only 9.18 percent of the catch. The cutthroat catch peaked in August, possibly as a result of midsummer recruitment. The 6- to 8-inch size class of cutthroat was the most important class.

1942. Success of planting legal-sized trout in the Southwest. Transactions of the Seventh North American Wildlife Conference. pp. 238–245.

Plants of legal-sized rainbow and cutthroat trout were made from 1939 to 1941, and in spring, summer, fall, and winter plantings. Returns for the two species were generally comparable, with returns from 37.3 to 58.8 percent from spring and summer plantings. Best returns were from releases just before opening of fishing season and during the season. Overwintering of planted fish was poorer than with wild fish.

GILBERT, CHARLES H., and BARTON W. EVERMANN.

1894. A report upon investigations in the Columbia River Basin, with descriptions of four new species of fishes. Bulletin of the U.S. Bureau of Fisheries, 14: 169–204.

Discusses morphology of various subspecies of cutthroat in this drainage and others.

GRASSE, JAMES E.

1949. Beaver and trout. Wyoming Wildlife, 13(11): 4–13.

Review of work on beaver-trout relations is given. Beaver in Wyoming are discussed, and the place of cutthroat and brook trout in relation to these species is treated. The author concludes that beaver ponds are beneficial to trout in Wyoming, and that the brook trout is the beaver-pond trout in that State.

GREENE, WILLIAM S., Jr.

1937. Colorado trout. Colorado Museum of Natural History, Popular Series, No. 2. 48 pp.

Contains sections on *Trutta pleuriticus*, *T. virginalis*, *T. macdonaldi*, and *T. stomias*, with treatments on identification, distribution, and biology.

GRIFFIN, L. E.

1938. Experiments on the tolerance of young trout and salmon for suspended sediment in water, In "Placer mining on the Rogue River, Oreg., in its relations to the fish and fishing in that stream." Oregon Department of Geology and Mineral Resources, Bulletin 10, Appendix B: 28–31.

Experiments were performed to learn the direct effects of soil sediment in water on fish. The fish were 2- to 2½-inch cutthroat trout and young chinook salmon. Some cutthroat were held in troughs and 2,300 to 3,500 p.p.m. of sediment was added to the flowing water; other cutthroat were held in clear water. The fish in clear water were nervous during the experiment, while those in cloudy water were bold. After 2 weeks, 56 percent of the trout in the cloudy water were alive; 10 percent of the control fish were alive. With salmon, in water containing 3,100 to 6,500 p.p.m. of sediment, 88 percent were alive after 28 days; control salmon had a 36 percent survival in the same time. The experiment shows that cutthroat trout and chinook salmon can live in muddy and opaque water, and can feed and grow well in very muddy water.

GRIFFITHS, FRANCIS P., and ELDEN D. YOEMAN.

1940. A comparative study of Oregon coastal lakes from a fish management standpoint. Proceedings of the Sixth Pacific Science Congress of the Pacific Science Association, 3: 323–333.

Oregon Game Commission studied certain coastal lakes in Oregon to compare productivity and to find a brood lake for coastal cutthroat. Geographical and climatological features of the lakes are discussed, fish faunas of 10 lakes are listed, and their limnology treated. Food of cutthroat of the lakes is composed mostly of insects. Condition factors ranged from 41.67 to 69.47. Suggestions for management of the lakes are presented.

HAGEN, HAROLD K.

1958. A case for the native. Wyoming Wildlife, 22(9): 31–37.

This article describes the dominance of rainbow and brook trouts over the cutthroat in the Rocky Mountain region. The life history of the cutthroat is described in detail.

HAIG-BROWN, RODERICK L.

1939. The western angler—an account of Pacific salmon and western trout. Derrydale Press, New York. 200 pp.

Good account in nontechnical language of biologies and life histories of cutthroats in British Columbia. Chapter on coastal cutthroats describes migrations in detail, and another on nonmigratory cutthroat contains a great deal of valuable material on habits.

HALL, E. RAYMOND.

1924. Pelicans versus fishes in Pyramid Lake. Condor, 27 : 147–160.

Importance of cutthroat trout of Pyramid Lake is discussed, and the decline of commercial and sport fisheries is described. Investigation of white pelicans in relation to cutthroat was begun in 1924. Food habits of pelicans and their feeding behavior in herding trout are treated. Red suckers, lake minnows, lake chubs, carp, Sacramento perch, and catfish were taken by the pelicans, as well as cutthroat up to 8 pounds. Trout were considered accidental food items. It is concluded that the white pelican is not detrimental to fishing industry, although local people believe it eats large numbers of cutthroat. Decline of trout is blamed on depletion of water, as well as on a dam, diversions, and pollution.

HALL, MAURICE C.

1930. Report on parasites of pelican and trout investigations at Yellowstone Lake, 1930. Unpublished report in Yellowstone Museum. 10 pp.

Studies on tapeworms in cutthroat and pelicans featured postmortem examination of pelicans for parasites and antihelminthic treatment of pelicans. Pelicans are freed from tapeworms with kamala. Relation between fish size and parasitism is discussed, as is the relation between parasitism and reduced cutthroat egg production. Antihelminthic treatment of pelicans is recommended as a control for tapeworms in Yellowstone Lake cutthroat.

HAMMER, GLEN L.

1960. Toxicity of pyridylmercuric acetate to cutthroat trout. Progressive Fish-Culturist, 22(1) : 14.

Two-year-old Snake River cutthroat were treated for 1 hour with PMA at a concentration of 1 : 500,000 and returned to rearing tanks. The treatment was repeated the next day. Two hours after the second treatment they were in distress and mortality was 24 percent after 24 hours.

Brown, brook, rainbow, and cutthroat, 3 to 18 inches in length, were then given the same treatment, the larger cutthroat being survivors from the first test. After 3 days, the lake, brown, brook, and larger cutthroat showed 100 percent survival. Cutthroat fingerlings suffered almost 25 percent mortality.

HANZEL, DELANO A.

1961. The distribution of the cutthroat trout (*Salmo clarki*) in Montana. Proceedings of the Montana Academy of Sciences, 19 : 32–71.

A study was made to determine distribution and relative abundance of pure cutthroat trout stocks in Montana and to secure information on factors that influence distribution and abundance. Observations were also made on taxonomic differences between strains and on the prevalence of hybrids. Barriers in relation to cutthroat distribution were noted. Exotic fish planted above barriers were predominant. Details of distribution of cutthroat and other fish are given in tabular form for hundreds of Montana lakes and streams.

HARTMAN, GORDON F.

1956. A taxonomic study of cutthroat trout, *Salmo clarki clarki* Richardson, rainbow trout, *Salmo gairdneri* Richardson, and reciprocal hybrids. Master's thesis, University of British Columbia. 71 pp.

Reciprocal hybrid crosses were made of cutthroat and rainbow trout, and the eggs were reared under similar conditions with pure cutthroat and pure rainbow eggs. Viability of hybrid crosses was compared with viability of pure strains, and sex ratios in offspring were studied. Relative growth of several head and body parts was studied in the four lots of fish. Meristic comparison, counts of teeth, and of pyloric caecae, were made. Coloration was studied in all experimental lots of fish. Hybrid crosses were as viable as interspecific crosses, and sex ratios did not differ significantly from those expected. Relative growth studies showed cutthroats had larger heads, larger head parts, and deeper peduncles than rainbows. Hybrids were usually intermediate. Rainbow trout had higher scale, ray, and vertebral counts than cutthroats. Contrary to most authorities, scale counts were higher on rainbows than on cutthroat. Dorsal ray counts for both hybrid lots resembled rainbow parents. Vertebral counts among hybrids tended to resemble female parents. No marked differences were found in teeth except on the hyoid bone. Pyloric caeca counts were similar in both parental lots. Coloration on rainbows was different from that on cutthroats. Hybrids were intermediate in some aspects of color, and in others they tended to resemble one parent. Eight samples of wild fish were examined, and several features which were distinctive in the hatchery fish were different for the two species in the wild.

Hatton, S. Ross.

1932. The fish fauna of Utah Lake. Master's thesis, Brigham Young University, Provo, Utah. 64 pp.

Synonymy, description, and habits of *Salmo utah* are given. Historical importance of this fish in Utah Lake is discussed. Gradual disappearance of the species from the Great Basin is described.

Hazzard, A. S.

1932. Preliminary report of a biological survey of the streams and lakes of Grand Teton National Park and Jackson Lake, Wyoming. Unpublished report to the U.S. Bureau of Fisheries. 94 pp.

The cutthroat is treated at some length. Average size and condition factors are given for many waters. Spawning habits and distribution are described for Teton area. Average condition factor for all lakes was 1.345, and for all streams, 1.328.

Hazzard, A. S., and M. J. Madsen.

1933. Studies of the food of the cutthroat trout. Transactions of the American Fisheries Society, 63 : 198–207.

Food studies of *S. lewisi* from Jackson Lake, Wyo., Glacier Park, Mont., and other lakes and streams in Teton Park, Wyo., were conducted over three seasons. Diet of smallest fingerlings is diversified, midges being most important item. Considerable variation in diet was seen from stream to stream and from lake to lake. Change in diet at 11½ inches was noted in Jackson Lake fish from crustacea toward fish. A variety of fish species was utilized. Large (11¾ to 14½ inches) cutthroat from Strawberry Reservoir, Utah, had only midge larvae and pupae in their stomachs. Stomachs of *Salmo gibbsii* from Middle Fork of Salmon River in Idaho had great numbers of snails, with fish, fish eggs, caddis flies, and stoneflies. Cutthroat diets are similar to those of other trouts.

Henshall, James A.

1902. Food and game fishes of the Rocky Mountain region. Transactions of the American Fisheries Society, 31 : 74–88.

Several common names for cutthroat in the Rocky Mountain region are given. Distribution and habits are discussed.

1906. A list of the fishes of Montana. Bulletin of the University of Montana, No. 34, 13 pp.

Red-throat trout is discussed with reference to distribution, color patterns, and habits.

Hildebrand, Samuel F., and Irving L. Towers.

1927. Food of trout in Fish Lake, Utah. Ecology, 8(4) : 389–399.

Contents of 181 stomachs of brook, lake, rainbow, and cutthroat trout were studied with reference to physical conditions and plant and animal life of Fish Lake, Utah. *Daphnia*, amphipods, insects, and *Nostoc* colonies were chief organisms found. Lesser numbers of fish eggs, *Pisidium*, snails, and certain higher plants were found.

Hochachka, P. W.

1961. Liver glycogen reserves of interacting resident and introduced trout populations. Journal of the Fisheries Research Board of Canada, 18(1) : 125–135.

Three groups of trout, two introduced populations of *Salmo gairdneri* and a resident *Salmo clarki*, were studied in stream sections. Liver glycogen deposits, which were reduced to low levels during transportation to the stream, were restored in 2 to 3 weeks in all groups, with recovery rates being approximately inverse to the population density. Within the hatchery groups, larger fish laid down greater glycogen stores. Wild trout maintained their high glycogen reserves throughout the experiment.

1963. On the need for measuring the buffering capacity of fish blood. Journal of the Fisheries Research Board of Canada, 20(3) : 845–847.

In this study on fish blood buffering capacity, concentrations of hemoglobin in cutthroat and rainbow trout from the wild were compared with those of several groups of hatchery rainbow. Cutthroat averaged 12.0 g. hemoglobin per ml. of blood for an 8–72 gram group, and 11.3 for a 72–154 gram group. Wild rainbow averaged 11.9 g. for an 18–80 gram group. Hatchery-reared fish generally averaged lower values than those for wild fish.

Hochachka, P. W., and A. C. Sinclair.

1962. Glycogen stores in trout tissues before and after stream planting. Journal of the Fisheries Research Board of Canada, 19 (1) : 127–136.

Changes in the glycogen reserves of epaxial and heart muscle of rainbow trout were followed after stream planting. Muscle glycogen recovered quickly in large fish, more slowly in smaller ones, and was related to earlier reported changes in liver glycogen and blood lactic acid. Heart glycogen increased initially, but fell again shortly after feeding became stabilized. Muscle glycogen reserves of wild cutthroat trout were lower in the presence of hatchery fish than in their absence. A depletion of some metabolite, such as glycogen, in conjunction with an increased body demand due to raised basal metabolism was suggested as a factor in delayed mortality.

Holmes, W. N., and Gael H. Stott.

1960a. Studies of the respiration rates of excretory tissues in the cutthroat trout (*Salmo clarki clarki*). I. Variations with body weight. Physiological Zoology, 33(1) : 9–14.

Oxygen consumptions of kidney and gill tissues of cutthroat trout (*Salmo clarki clarki*) were examined in relation to body weight. A decline in weight specific oxygen consumption with increase in body weight for both tissues was observed (kidney —0.148, gill —0.139). This decline was not significantly different for the two tissues.

The regression coefficients of Q_{O_2} values for kidney and gill tissue (—0.148 and —0.139) were statistically compared to a theoretical value of —0.27, which would correspond to the 0.73 rule. Such comparison showed a highly significant difference (p 0.005, 0.001), indicating that the decline in metabolism of these tissues with increasing body weight is not according to the 0.73 rule.

1960b. Studies of the respiration rates of excretory tissues in the cutthroat trout (*Salmo clarki clarki*). Physiological Zoology, 33(1) : 15–20.

Cutthroat trout (*Salmo clarki clarki*) were transferred from fresh water to 65-percent standard sea water. The oxygen consumptions of kidney and gill tissues were examined during a 168-hour period after transfer.

A sharp initial rise in Q_{O_2} of kidney tissue was noted during the first 48 hours after transfer, reaching a maximum at 20 hours. The tissue respiration during the rest of the experimental period remained significantly higher than the parallel control level.

The gill-tissue respiration declined rapidly during the first 10 hours after transfer and remained significantly below the control level for the whole experimental period.

The results are discussed in relation to recent observations concerning possible hormonal regulation in euryhaline species of salmonoids.

Huey, William S.

1956. New Mexico beaver management. New Mexico Department of Game and Fish, Bulletin No. 4. 49 pp.

During summer of 1955, studies were conducted to determine beaver-trout relations. Two inventories were made in each of six experimental sections of upper Red River in Taos County. Three of these contained beaver ponds and three did not. Each section had 50 yards of stream. Cutthroat, brook, and rainbow trout and white suckers were in the experimental area. Average trout population in beaver sections was 154 in June and 180 in August, as opposed to 33 in June and 47 in August for nonbeaver sections. Trout from beaver ponds were slightly larger than those from nonbeaver sections. Food production was significantly higher in beaver sections and trout growth appeared to be faster in these sections.

Huey, William S., and William H. Wolfrum.

1956. Beaver-trout relations in New Mexico. Progressive Fish-Culturist, 18(2) : 70–74.

Effect of beaver activity on trout production in Red River, N. Mex., was studied. Cutthroat, rainbow, and brook trout were present; cutthroat and brook amounted to 98–99 percent of the trout.

Production was increased in beaver ponds. Ponds produced more trout than undisturbed stream areas, and growth was faster. Cutthroat were apparently not prevented from spawning because of beaver activity.

Idyll, Clarence.

1942. Food of rainbow, cutthroat, and brown trout in the Cowichan River System, B.C. Journal of the Fisheries Research Board of Canada, 5(5) : 448–458.

Stomach contents of 293 rainbow, 160 cutthroat, and 113 brown trout were examined and analyzed according to species, size groups (5 cm.), and habitat (river or lake). For rainbows of both river and lake (4 to 50 cm. in length), insects, chiefly Trichoptera and Simuliidae, were predominant in all size groups. Fish constituted a small fraction of the food except in winter when salmon eggs were available. River cutthroat subsisted chiefly on insects (Trichoptera) up to 15 cm., on insects and fish (*Gasterosteus*) up to 30 cm., thereafter largely on fish. Lake cutthroat did not eat fish in any number until 41 cm. long. Brown trout ate chiefly insects (Trichoptera) up to 45.5 cm., thereafter turning more definitely to a fish (salmonid) diet. Cutthroat were more piscivorous than brown trout. Definite selection of food by the trout was indicated.

Irving, Robert B.

1953. Ecology of the cutthroat trout, *Salmo clarkii* Richardson, in Henrys Lake, Idaho. Master's thesis, Utah State Agricultural College. 101 pp.

Cutthroat trout of several stocks live in Henrys Lake with rainbow and brook trouts, whitefish, bullhead, and silverside minnow. Limnological data suggest high productivity. Growth is rapid, and body-scale relation is 21.59 mm.+6.879 S_t+0.03874 S_t^2. Only 2 percent of cutthroat reach 5 years of age; 2 percent of rainbow and cutthroat hybrids reach 7 years of age. Length-weight relation of cutthroat is $Log\ W = -4.6353 + 2.9529\ Log\ L$. Average female cutthroat has 2,703 eggs. Average total length of spawners is 18.5 inches for male and 19 inches for female. Sex ratio in 1951 was 96 males to 100 females.

Potential harvest is estimated at 89.2 pounds per surface acre. In 1951 the lake yielded 5.1 pounds per surface acre. Cutthroat in the creel averaged 2.5 pounds.

1956. Ecology of the cutthroat trout in Henrys Lake, Idaho. Transactions of the American Fisheries Society, 84: 275–296.

Henrys Lake in eastern Idaho is a shallow, fertile lake, with an estimated 606 pounds of bottom and plant-inhabiting fauna in 1951. *Salmo clarki, Cottus bairdi semiscaber*, and *Prosopium williamsoni* are the native fish; *Salmo gairdneri, Salvelinus fontinalis*, and *Richardsonius balteatus hydrophlox* are introduced species. Rainbow-cutthroat hybrids were rare in the catch. Body scale relation in cutthroat was $L = 21.59 + 6.879\ R + 0.03874\ R^2$. Male cutthroat in 1951 matured at ages II and III, females at ages III and IV. Severe mortality subsequent to spawning is reported for 1951. Variation in growth is attributed to differences between time spent in streams and in the lake, seasonal variation of environmental conditions, and different ages at maturity. Rates of female to male cutthroat in catch decreased with age and progress of season. Ninety percent by number and 74 percent by volume of food were midges, shrimp, and damsel flies. Pathogenic bacteria infected mature fish in 1950, 1951, and 1952 spawning migrations. Asphyxiation was cause of mortalities at mouths of streams in January 1952. The population is short lived owing to disease, asphyxiation, predation, angling, and spawning mortality.

Jeppson, Paul W., and William S. Platts.

1959. Ecology and control of the Columbia squawfish in northern Idaho lakes. Transactions of the American Fisheries Society, 88(3): 197–202.

Columbia squawfish, *Ptychocheilus oregonensis*, and trout were found to occupy similar niches, resulting in competition for food and space, and predation on each other. Mature squawfish are piscivorous, with food habits similar to Dolly Varden, *Salvelinus malma*, rainbow trout, *Salmo gairdneri*, and brown bullhead, *Ictalurus nebulosus*. Age and growth data are presented; in northern Idaho lakes a 13-year-old female squawfish attains a maximum weight of approximately 9 pounds. Males grow slower, averaging less than one-half the size of the females. Spawning by lake populations occurs in lakes and streams. Lake spawning is in schools during late May and early June over wave-washed rubble on clear, calm days. The number of eggs in mature females varies from 12,000 to 100,000, averaging 40,000. Eggs hatch in 7 to 8 days. In 6 years of squawfish control in 3,800-acre Hayden Lake by dynamiting and gill netting spawning schools, mature squawfish were reduced to about one-tenth of their original abundance and an increase in trout was noted.

Food habits of cutthroat trout are presented in tables.

Johnson, A. K.

1961. Ichthyophthiriasis in a recirculating closed-water hatchery. Progressive Fish-Culturist, 23(4): 70–82.

Cutthroat and other salmonids were infested with *Ichthyopthiriasis* at the Humboldt State College hatchery in 1958. The pathogen was apparently introduced from other stations. Some parasites were not stopped by filters. Treatment with salt and malachite green brought the infestation under control.

Johnson, Harlan E., and Richard F. Brice.

1952. Observations on columnaris in salmon and trout. Progressive Fish-Culturist, 14(3): 104–109.

Salmonids were reared in water from three levels in Dorena Reservoir in Oregon. No lesions of columnaris disease were seen in any fish in water from the lowest level, where the water was coolest. Columnaris lesions were observed in cutthroat and rainbow trout and king salmon, but not in silver salmon, in water that was near 60° most of the summer. Columnaris caused high mortalities in all salmon and trout reared in water from the high

level, where temperatures were above 65° all summer.

Sulfamerazine in the diet was beneficial to silver salmon and rainbow trout, but not to king salmon or cutthroat trout.

1953. Further observations on columnaris in salmon and trout. Progressive Fish-Culturist, 15(4): 183–185.

Columnaris in salmon and trout was studied at Cottage Grove, Oregon. Cutthroat trout reared in hatchery troughs suffered mortality from columnaris in water above 65° F; sulfamerazine fed 10 to 13 weeks effectively controlled the disease.

JORDAN, DAVID STARR.

1890. The fishes of the Yellowstone Park. Zoe, 1(2): 38–40.

Discussion of native fishes of Yellowstone Park. Describes historical geology of area and suggests that *Salmo mykiss* entered Yellowstone Lake and River from Pacific Creek in Snake River drainage.

1891a. Report of explorations in Colorado and Utah during the summer of 1889, with an account of the fishes found in each of the river basins examined. Bulletin of the U.S. Fish Commission, 9: 1–40.

Discussions of distribution, appearance, and biology of *S. m. macdonaldi*, yellow-finned trout; *S. m. stomias*, green-backed trout; *S. m. clarki*, cutthroat trout; *S. m. pleuriticus*, Colorado River trout; *S. m. spilurus*, trout of the Rio Grande; *S. m. virginalis*, trout of Utah Lake; *S. m. bouvieri*, Waha Lake trout; *S. m. henshawi*, Lake Tahoe trout; *S. m. lewisi*, trout of the Upper Missouri.

1891b. A reconnaissance of the streams and lakes of the Yellowstone National Park, Wyoming, in the interest of the United States Fish Commission. Bulletin of the U.S. Fish Commission, 9: 41–63.

Discusses distribution of each drainage. Much detail is given on distribution of *S. mykiss* and infestation by *Dibothrium* worms. Classified list of waters suitable for *S. mykiss* is presented.

1907. The trout and salmon of the Pacific Coast. State Board Fish Commission of California, Report (1905–1906): 77–92.

Describes discovery of cutthroat in Oregon by Clark. Movements of trout species in northern hemisphere are described; movement of cutthroat from Alaska southward is traced. Color patterns are given. Tahoe trout and Crescent trout are treated separately.

1920. The trout of the Rio Grande. Copeia, No. 85. pp. 72–73.

Nomenclature of cutthroat is discussed. *Salmo virginalis* is the Rio Grande trout, and *S. utah* is the Bonneville Basin trout.

1928. The distribution of fresh-water fishes. Annual Report of the Smithsonian Institution for 1927. pp. 355–385.

Principles of zoogeography are discussed, including methods of distribution of fishes. Geographic barriers throughout world are described, with their influence on groups of fishes. Dispersal of fresh-water fishes is discussed in general terms and movement of cutthroat over Two-Ocean Pass in Wyoming is treated in detail. Speciation in cutthroat in Great Basin is described.

JORDAN, DAVID STARR, and H. W. HENSHAW.

1879. Report upon the fishes collected during the years, 1875, 1876, and 1877, in California and Nevada. Report of the Chief of Engineers, Part 3, Appendix NN, Appendix K, pp. 1609–1622. Executive Documents, 3d Sess., 45th Cong., 1878–79, vol. 5.

Contains detailed descriptions of *S. tsuppitch* Richardson (black trout), *S. henshawi* Gill & Jordan (silver trout), and *S. pleuriticus* Cope (Rio Grande trout), with notes on distribution and biologies.

JORDAN, DAVID STARR, and BARTON W. EVERMANN.

1902, 1934. American food and game fishes. Pp. 175–179. Country Life Press, Garden City, N.Y.

Keys to species of cutthroat are given. Discussion of distribution, biology, and habits of *S. clarki*, *S. lewisi*, *S. gibbsii*, *S. henshawi*, *S. tahoensis*, *S. utah*, *S. jordani*, *S. bathoecetor*, *S. declivifrons*, *S. virginalis*, *S. pleuriticus*, *S. bouvieri*, *S. stomias*, and *S. macdonaldi* is presented.

JUDAY, CHAUNCEY.

1907a. Notes on Lake Tahoe, its trout and trout-fishing. Bulletin of the U.S. Bureau of Fisheries, 26: 133–146.

Description of Lake Tahoe, its vegetation, and its plankton is given. Account of *Salmo henshawi* (Lake Tahoe trout, Truckee trout, pogy, and snipe) and *Salmo tahoensis* (silver trout) is presented. Gives distribution, breeding habits, hatchery operation, food habits, angling methods, shipments of trout from the lake, and their commercial value ($6,819 in 1906).

1907b. A study of Twin Lakes, Colorado, with especial consideration of the food of the trouts. Bulletin of the U.S. Bureau of Fisheries, 26: 147–178.

Describes physical characteristics of the lakes, aquatic vegetation, plankton, and fishes, including *Salmo macdonaldi* (yellow-fin trout) and *Salmo stomias* (green-back trout). Importance of study of fish food is discussed, and details of analyses of fish stomachs are given. *S. stomias* stomachs contained fish remains, insects, crustaceans, and vegetable fragments. Importance of plankton crustacea as fish food is discussed at length.

KEMMERER, GEORGE, J. R. BOVARD, and W. R. BOORMAN.

1924. Northwestern lakes of the United States; biological and chemical studies with reference to possibilities in production of fish. Bulletin of the U.S. Bureau of Fisheries, 49 : 51–140.

Physical and biological surveys were made in many northwestern lakes for comparisons with each other and with Wisconsin lakes, and to determine suitability for trout. Cutthroat trout found in Bear Lake, Henrys Lake, Coeur d'Alene Lake, Hayden Lake, Payette Lake, Pend Orielle Lake, Priest Lake, and Spirit Lake, (all in Idaho). Food habits are discussed.

KENDALL, W. C.

1915. The fishes of the Yellowstone National Park. Report of the U.S. Commissioner of Fisheries for 1914, App. 8. pp. 1–28.

Fishing for cutthroat and other species is described for major waters of Yellowstone Park. Tapeworm parasite of cutthroat of Yellowstone is discussed. Detailed distribution of cutthroat is given for Yellowstone Park. Distribution from hatchery is listed.

1920. What are rainbow trout and steelhead trout? Transactions of the American Fisheries Society, 50 : 187–199.

The difficulty in classifying *Salmo mykiss* is discussed. Connections between Asian and North American cutthroat are described. The identities of resident rainbow and steelhead are discussed.

LAAKSO, MARTIN.

1955. Variability in scales of cutthroat trout in mountain lakes. Proceedings of the Utah Academy of Science, Arts, and Letters, 32 : 81–87.

Some cutthroat trout in mountain lakes fail to establish a year mark during first year of life and have scales designated as "retarded." Fry which are more advanced in their first fall have year marks corresponding to each year of life and are called "normal." Frequency of normal scales was found to be closely correlated (r.=.79) with growth index (quotient of mean length divided by mean age for each sample) in total of 12 areas.

1956. Body-scale regressions in juvenile cutthroat from Yellowstone Lake. Proceedings of the Utah Academy of Science, Arts, and Letters, 33 : 107–111.

The body-scale regressions of juvenile cutthroat trout from different areas in Yellowstone Lake showed significant differences in slope. Curves plotted for fingerlings captured near the outlet of Yellowstone Lake exhibited steeper slopes, showing that scale growth was relatively faster than in fish collected along the lakeshore and in two small tributaries.

LAAKSO, MARTIN, and OLIVER B. COPE.

1956. Age determination in the Yellowstone cutthroat trout by the scale method. Journal of Wildlife Management, 20(2) : 138–153.

Fishery studies on cutthroat trout in Yellowstone Lake have required investigation into technique of age determination. Scales exhibit growth characteristics different from those of same species in other waters at lower altitudes. Present studies have centered around identification of scales on which first annulus failed to form.

Cutthroat trout in Yellowstone Lake ascend spawning streams from May to late July. Fry emerge from late June to early September. Scale formation is initiated when fish are 41 to 44 millimeters in length. Late-hatched fry may not attain this length before interruption of growth in October, and thus may overwinter without scales or with only a few small scales. As a result, three developmental types of fry occur in the population: (1) fry overwintering in a fully scaled state; (2) fry passing the winter partially scaled; and (3) fry overwintering without scales.

Annulus development after first growing season occurred on scales of fingerlings whose scale growth had reached 4-circulus stage. Late-hatched fry with rudimentary scales failed to establish a year mark during their first fall and winter period. These fish with "retarded" scales exhibit annulus formation following their second growing season. Term "retarded scale" is proposed to designate a scale that does not exhibit an annulus after first growing season, and "normal" is applied to a scale that did establish a year mark after one season's growth. Structural criteria have been established for identification of the two types of scale development. Bases for differentiating the types are (1) numbers of circuli before first annulus, (2) size of focus, or first

circulus, and (3) shape and density of first one or two circuli.

Annulus formation begins before May 1 and is usually complete by middle of June. Spawners appear to initiate scale growth before nonspawners.

Fingerlings taken late in season and presumed to be in first year of life included 35 percent fully scaled individuals, unscaled comprised 22 percent, partially scaled made up 42.7 percent. Adults sampled from 1951, 1952, and 1953 fisheries averaged 40 percent normal.

LAND, S. E.

1913. The black-spotted mountain trout. Transactions of the American Fisheries Society, 42 : 183–198.

Salmo stomias Cope and *Salmo spilurus* Cope are discussed as to distribution and handling.

LEACH, GLEN C.

1923. Artificial propagation of brook trout and rainbow trout, with notes on three other species. Report of the U.S. Comissioner of Fisheries for 1923, App. 6. pp. 1–74.

Describes places where cutthroat trout have been cultured. Spawning season, growth, and egg production are noted. Quotes articles relating to biology of this species. Tapeworm parasite at Yellowstone is discussed.

LINTON, EDWIN.

1891a. On two species of larval dibothria from Yellowstone National Park. Bulletin of the U.S. Fish Commission, 9 : 65–79.

A collection of *Salmo mykiss* and suckers from Yellowstone Park were examined for parasites. Larval cestodes were found in both fishes. Detailed descriptions of anatomies of the tapeworms are given, and figures are presented. *Dibothrium cordiceps* Leidy is the worm found in the cutthroat.

1891b. A contribution to the life history of *Dibothrium cordiceps* Leidy, a parasite infesting the trout of Yellowstone Lake. Bulletin of the U.S. Fish Commission, 9 : 337–358.

Discussions of larval and adult stages of *D. cordiceps* from *Salmo mykiss* in various waters of the Yellowstone and an account of distribution and anatomy and of pelican hosts. Author accounts for abundance of parasitized trout in Yellowstone Lake and migration of parasite into muscular tissue of its host.

1893. On fish entozoa from Yellowstone National Park. Report of the U.S. Commissioner of Fisheries 17 : 545–564.

This paper treats all internal parasites found in a collection of trout, suckers, and chubs from Yellowstone Park. Parasites from *Salmo mykiss* were : tapeworm *Dibothrium cordiceps* Leidy, fluke *Distomum laureatum* Zeder, acanthocephalan *Echinorhynchus globosus* Rud, and nematode *Dacnitis globosa* Dujardin. Details of life history and biology are given for some of these parasites.

LOCKE, S. B.

1929. Whitefish, grayling, trout, and salmon of the intermountain region. Report of the U.S. Commissioner of Fisheries for 1929, App. 5. pp. 173–190.

Identification and description of salmonids in intermountain region are treated. Cutthroat is discussed in the various forms reported for drainages in this region.

LORD, RUSSELL F.

1930. Rearing a brood stock of blackspotted trout. Transactions of the American Fisheries Society, 60 : 164–166.

Sources of blackspotted trout eggs are suffering a decline in abundance, and it seems necessary to rear new brood stocks of this species. Attempts in 1920–22 at Springville, Utah, were unsatisfactory. Experiments at Pittsford, Vt., beginning in 1926, resulted in an egg take of 115,000 eggs in 1930. An 8.6-percent loss was suffered up to the eyed stage. Shipments were made, and fry were feeding nicely at latest report.

MADSEN, DAVID H.

1936. Protection of native fishes in the National Parks. Transactions of the American Fisheries Society, 66 : 395–397.

Planting policies in national parks are discussed, and rainbow-cutthroat hybridization through planting is described. Importance of this trend in Rocky Mountains is stressed. Policy for management of cutthroat and other native species in national parks is presented.

MADSEN, M. J.

1940. Report on age and growth of the cutthroat trout (*Salmo lewisi*) of Yellowstone Lake, Wyoming. Unpublished report of the U.S. Fish and Wildlife Service. 12 pp.

Studies on scales collected from Yellowstone Lake in 1938 and 1939 showed age group III to dominate the fishery, followed by age groups IV, II, and V. Sexes in catch were 54.8 percent males and 45.2 percent females in 1938; in 1939, percentages were 67.4 and 32.6, respectively.

Condition factors were calculated as 1.2271 for 1938 and 1.3027 for 1939. Condition factors for males were higher than for females.

Weight-length relations are presented.

Observations on parasitism by tapeworms were made, showing that in 1938, 31.8 percent of the fish examined were parasitized; in 1939 the percentage was 45.7. Parasitized fish had lower condition factors than did nonparasitized ones.

Mason, James E.

1953. The distribution of four anadromous members of the genus *Salmo* in the northern hemisphere. Progressive Fish-Culturist, 15(2): 51–56.

Salmo clarki clarki is one of the four anadromous members of the genus *Salmo.* Spawning and migration times are discussed. Cutthroat range in colder waters than the other species. Air and sea surface temperatures are used as indexes of the environments. Maps of distributions and temperature tables are presented.

McConnell, William J., William J. Clark, and William F. Sigler.

1957. Bear Lake, its fish and fishing. Utah Department of Fish and Game, Idaho Department of Fish and Game, Wildlife Management Department, Utah State Agricultural College. 76 pp.

The cutthroat is one of three important game fish of Bear Lake. Methods of catching cutthroat are discussed, as well as importance of cutthroat in the fishery. Gill netting showed cutthroat to be most abundant between shore and 75-foot contour throughout year. Life history of cutthroat in Bear Lake is treated, and calculated total lengths to seventh year are given. Creel census showed that 900 to 1,000 cutthroat were caught annually from 1953 to 1956. History of planting cutthroat in Bear Lake is given. Length frequencies of cutthroat taken in creel are figured. It is concluded that present cutthroat population is only slightly exploited, and that if population density were increased, shore fishermen would probably not enjoy greatly increased success.

Merriman, Daniel.

1935. The effect of temperature on the development of the eggs and larvae of the cutthroat trout (*Salmo clarkii clarkii* Richardson). Journal of Experimental Biology, 12(4): 297–305.

Eggs of coastal cutthroat were raised from fertilization through hatching at constant temperatures of 11.3°, 8.25°, and 6.35° C. Temperature is not limiting factor in determining length of hatching period. Average size of cutthroat embryos at hatching was smaller at higher temperatures and larger at lower temperatures. Maximum increment of growth occurred about 41st day at 8.25° C. and about 28th day at 11.3° C. Percentage of dry weight of cutthroat embryos showed a steady decrease from about 25 to 14 percent, while that of yolks showed an increase from about 46 to 55 percent. Embryos absorbed water from the yolk, and at a faster rate from the environment, both before and after hatching.

Miller, Richard B.

1952. Survival of hatchery-reared cutthroat trout in an Alberta stream. Transactions of the American Fisheries Society, 81: 35–42.

Gorge Creek, typical small mountain trout stream in southwestern Alberta, was divided by fish-tight screens into two sections, each about three-quarters of a mile in length. The number of native trout in these was determined by marking and recapture. One section was left as a control. Into the other, 199 3-year-old and 201 2-year-old hatchery-reared cutthroat trout were liberated. Each of the planted trout was weighed and given a numbered Petersen tag before planting. Fish that died were removed daily and weighed. Daily angling was carried on to recapture, weigh, and again release as many as possible. One-third of the 3-year-old fish died, mainly in the first 2 weeks following planting. All lost weight for 40 days, apparently through inability to compete with the native population. By fall (about 100 days after planting) the survivors had regained their planted weights. All, or nearly all, of the 2-year-old fish died, apparently from a combination of shock and starvation. Native trout in experimental section gained weight all summer, and by fall were in as good condition as native trout in control section.

Plans for planting hatchery-reared trout in sections of stream free of native trout and for transplanting native trout into sections containing native trout are described.

1954a. Movements of cutthroat trout after different periods of retention upstream and downstream from their homes. Journal of the Fisheries Research Board of Canada, 11(5): 550–558.

A homing experiment of cutthroat trout (*Salmo clarki*) was carried out in Gorge Creek, a small trout stream more or less typical of streams of eastern slopes of Rocky Mountains in Alberta. A half-mile portion of the stream was screened off and, into the enclosure thus formed, 105 trout were transferred from above and 104 from below. Each of these was given

a numbered tag and the home locality was recorded. Transfers were made from June 24 to August 13. On August 15 the screens were removed; September 3 to 5, most of the experimental area was poisoned with Fish Tox and the localities of tagged fish recorded. It was found that most of the trout that had been confined for 30 or more days remained in the enclosure area, i.e., they had adjusted to new homes. Exceptions appear to be result of random movements. Trout of upstream origin, confined for less than 30 days, move toward their original home territory. Trout of downstream origin, confined for less than 30 days, show very much less ability to move toward or to find their homes. Hypothesis is that trout are guided by smell in finding home.

A few observations on size of home territory suggest that it is small, perhaps not more than 20 yards of stream. Trout may inhabit same area of stream for at least 3 years.

1954b. Comparative survival of wild and hatchery-reared cutthroat trout in a stream. Transactions of the American Fisheries Society, 83 : 120–130.

Gorge Creek, typical small mountain trout stream on eastern slope of Rocky Mountains, was used in test to measure survival and weight changes in hatchery-reared cutthroat trout (*Salmo clarki*). Resident population of this species exists in the stream. Experimental procedure was to introduce groups of trout into enclosures one-half to three-fourths of a mile long; each trout in a group was given a numbered Petersen tag and weighed before planting. Recapture by angling and reweighing was carried out throughout the season of planting and also in later summers. In this way six lots of pond-reared, one lot of stream-reared, and one lot of transplanted wild cutthroat trout were studied. Pond-reared fish exhibited very low survivals over first (0 to 4.9 percent) and second (0 to 3.1 percent) winter. Survival was largely independent of age. Transplanted wild trout showed survivals of 46.0 to 29.0 percent to second and third summers, respectively. Stream-reared hatchery fish gave an intermediate value (17.2 percent to second summer).

All lots of trout lost weight for some 30 to 40 days when superimposed on a resident population. This loss was more severe and was regained more slowly in pond-reared trout than in transplanted wild trout.

It is held that low survivability of hatchery fish is due to absence of natural selection at early stages in life history.

1955. Trout management research in Alberta. Transactions of the Twentieth North American Wildlife Conference. pp. 242–252.

Experiments were conducted in Gorge Creek in South Saskatchewan River system to learn what happened to hatchery-reared cutthroat trout released in the stream.

With pond-reared cutthroat planted in an area with wild trout, there was an early, heavy mortality, followed by a period in which survivors lost weight. With wild cutthroat transplanted to an area with other wild cutthroat, the wild fish had lower mortalities and less weight loss than the pond fish.

Partially stream-reared cutthroat planted in the stream with wild fish suffered greater mortality than the wild, transplanted fish, but less than the pond-raised fish.

Hatchery-reared cutthroat planted in a stream with no resident population survived well and immediately began to gain weight. All groups showed low survival over first winter.

1957a. Permanence and size of home territory in stream-dwelling cutthroat trout. Journal of the Fisheries Research Board of Canada, 14(5) : 687–691.

Gorge Creek is a small cutthroat trout stream in Alberta on the eastern slopes of the Rocky Mountains. In the summer of 1954, 98 trout were tagged with Petersen tags, weighed, and returned to the pool of capture. In the same summer and in the next two, 58 of these trout were recaptured, some several times, so that, altogether, 83 records were obtained. Twenty-three records are of the first year, 35 from the second, and 25 from the third.

Sixty-seven percent of the recaptures were made in the original pool or less than 200 yards from it. Of the remaining 33 percent, one fish had moved 1.5 miles upstream and remained there. The others had all drifted downstream. The drifters were smaller fish than those that stayed home, and they either lost weight or gained at rates considerably below normal. It is suggested that these fish had been injured by the tags. The general conclusion is that each cutthroat trout of Gorge Creek has a home territory not over 20 yards long and that the whole life is spent in it.

1957b. Have the genetic patterns of fishes been altered by introductions or by selective fishing? Journal of the Fisheries Research Board of Canada, 14(6) : 797–806.

This paper is a review of some of the literature dealing with changes in fresh-water fish populations following exploitation or after introduction of exotic species. The object of the review was to look for changes that could be attributed to alterations in the gene pools of the affected populations. Very little concrete evidence could be found, either because the investigations reviewed were not looking for genetic changes, or because such changes did not occur. Changes in some salmonids may be assigned to introgressive hybridization, particularly where rainbow and cutthroat trout have been put together on the eastern slopes. In many cases, however, exotics have apparently failed to contribute to the gene pool of the resident population, and most hybrids, when they occur, have been of low fertility.

Fishes have a remarkable ability to respond to changes in population density by altered growth rates and times of maturity. These changed characters are not necessarily due to genetic changes and may be explained by assuming that fishes are preadapted to a wide range of conditions. There is a rather speculative suggestion that angling may select less intelligent fish, leading to an upgrading in intelligence of the fish. The general conclusion is that in the light of the existing evidence, great caution must be used in attributing to man-induced changes in fish populations changes in the genetic pattern.

1958. The role of competition in the mortality of hatchery trout. Journal of the Fisheries Research Board of Canada, 15(1): 27–45.

The literature on the survival of hatchery-reared trout after release in streams is reviewed, and the conclusion is reached that survival is poor in lakes and streams where a resident trout population already exists. In streams the deaths of planted trout occur very soon after their release and have been referred to as "delayed mortality." However, a comparison of survivals after planting in occupied and unoccupied streams shows that many of the deaths are not attributable to hatchery background or transportation methods, but largely to some aspect of competition with resident trout. Some investigations which have sought to measure the relative survivability of wild and hatchery trout have not used resident wild trout, and thus a crucial aspect of the competition has been omitted. Investigations at the Alberta Biological Station test stream, Gorge Creek, are described; in these a significant difference in blood lactic acid levels was found between hatchery trout with and without competition from resident trout. A tentative role is assigned competition as follows: introduced trout must compete for niches and for food. In the early stages of this competition they are continuously exercising; they exhaust stores of some metabolite and die either of acidosis or starvation.

Survival of wild cutthroat trout and of stream-reared hatchery trout and blood lactate levels in cutthroat trout are discussed.

Miller, Robert Rush.

1950. Notes on the cutthroat and rainbow trouts with the description of a new species from the Gila River, N. Mex. Ann Arbor, Mich.: University of Michigan Press. Occasional Papers, Museum of Zoology, No. 529. 42 pp.

Salmo gilae, a new species, is described. It resembles *S. gairdnerii* in some respects and *S. clarkii* in others. Status of trouts of western North America is reviewed. Comparisons between rainbow and cutthroat series are presented and discussed. Origins of *S. clarkii* are discussed.

Miller, Robert R., and J. R. Alcorn.

1946. The introduced fishes of Nevada, with a history of their introduction. Transactions of the American Fisheries Society, 73: 173–193.

History and distribution of *Salmo clarkii henshawi*, *S. c. lewisi*, and *S. c. utah* are described in detail for Nevada. Rainbow-cutthroat hybrids are also discussed, and stocking these species in the same water is discouraged.

Moore, H. L., O. B. Cope, and R. E. Beckwith.

1952. Yellowstone Lake trout creel censuses, 1950–1951. U.S. Fish and Wildlife Service, Special Scientific Report—Fisheries No. 81. 41 pp.

Two years of cutthroat trout creel census on Yellowstone Lake gave estimates of catch, effort, numbers of fishermen, and catch per hour for each unit of the fishery. Variances were calculated for catch and effort estimates, and mathematical basis for shoreline census method is described.

Catches of 1950 and 1951 were 199,993 and 207,860 for Yellowstone Lake. Catches per hour ranged from 0.16 to 2.12, depending on the unit of the fishery.

Morris, Robert W.

1950. An application of electricity to collection of fish. Progressive Fish-Culturist, 12(1): 39–42.

A lightweight, back-pack, DC shocker is described, and its use for collecting cutthroat trout is discussed.

MOTTLEY, C. McC.

1934. The origin and relations of the rainbow trout. Transactions of the American Fisheries Society, 64 : 323–327.

Rainbow and cutthroat forms of trout were evolving from a single salmonine stock in the last interglacial period after having been separated from progenitors of Pacific salmon by previous glaciation. Salmonine stocks were forced southward at last glaciation and then moved north again as ice melted. Cutthroats came into British Columbia from glacial Lake Bonneville and are found at higher elevations and farther from sea than rainbows.

1937. The number of vertebrae in trout (*Salmo*). Journal of the Biological Board of Canada, 3(2) : 169–176.

The European species of *Salmo* have fewer vertebrae than those from western North America, the mean values of two of each group as reared at the Cowichan hatchery being found to be: *salar* 59.0, *trutta* 58.3, *gairdnerii* 63.5, *clarkii* 62.5. Hatchery-reared *gairdnerii* were usually found to be different from those of the natural environment as well as varying with different experimental conditions, and even differed from their own parents, this seeming on the whole to be related to the temperature during development. A correlation was found between the length of the fish and the number of vertebrae. Caution is suggested in the use of the character for identifying populations of *Salmo*.

MUTTKOWSKI, RICHARD A.

1925. The food of trout in Yellowstone National Park. Roosevelt Wild Life Bulletin, 2(4) : 471–497.

The cutthroat and its environment, water food and surface food, feeding habits, and hints to anglers are discussed.

1929. The ecology of trout streams in Yellowstone National Park. Roosevelt Wild Life Annals, 2(2) : 155–240.

Contains detailed descriptions and classification of Yellowstone trout streams, with lists of flora and fauna. A little about biology of cutthroat itself is given.

NEAVE, FERRIS.

936. The development of the scales of *Salmo*. Transactions of the Royal Society of Canada, 30 (Third Series, Section V) : 55–72.

The microscopic development of scales in the genus *Salmo* is reviewed. Cutthroat, rainbow, and brown trouts were used during the study, and figures of scale formation in the lateral line area of cutthroat are given. The paper deals with distribution of the first-formed scales, development of scale papillae, differentiation of the papilla, laying down of the scale, the bony layer and the associated cells, and general considerations.

1943. Scale pattern and scale counting methods in relation to certain trout and other salmonids. Transactions of the Royal Society of Canada, 37 (Third Series, Section V) : 79–91.

The development of scale patterns in *Salmo clarki*, *S. gairdneri*, and *S. trutta* is described and discussed. Scale development begins after establishment of lateral line sense organs, and are metamerically distributed. Variations in number of scales are less extreme at the lateral line than at higher levels. Considerable differences between mean scale counts of different intraspecific populations occur. Lateral line counts will usually distinguish individual brown trout from individual cutthroats and steelhead. Cutthroats have a range of 116 to 133, rainbows a range of 114 to 131, and browns a range of variation of 12 scales at the lateral line. At higher levels on the body, cutthroats range from 122 to 177, rainbows from 115 to 159, and browns from 116 to 136.

1949. Game fish populations of the Cowichan River. Fisheries Research Board of Canada, Bulletin No. 84. 32 pp.

Some habits and life history of cutthroat in this British Columbia stream are described. Cutthroat are very widely distributed in this system. They spawn in winter and spring. Immatures may remain up to 2 years in spawning streams. Cutthroat move randomly in streams in winter, aside from movements connected with reproduction. Growth rate is variable. First spawning is usually at 3 or 4 years of age. Spawning more than twice is exceptional.

NEEDHAM, PAUL R., and F. K. CRAMER.

1943. Movement of trout in Convict Creek, California. Journal of Wildlife Management, 7(2) : 142–148.

A two-way fish trap was used in Convict Creek to study movements of brown, cutthroat, brook, and rainbow trout. Numbers of cutthroat trapped were small, and did not conclusively show migration patterns.

NEEDHAM, P. R., and ROBERT J. BEHNKE.

1962. The origin of hatchery rainbow trout. Progressive Fish-Culturist, 24(4) : 156–158.

In this discussion of the rainbow trout of the McCloud River system in California, the presence of cutthroat characteristics in Pit River populations is brought out. The idea is advanced that interior cutthroat entered the Pit River through an early connection with the Goose Lake drainage. Thus, there may be some cutthroat genes in the McCloud River rainbow, which furnished the original stock for hatcheries.

NEEDHAM, PAUL R., and RICHARD GARD.

1959. Rainbow trout in Mexico and California with notes on the cutthroat series. University of California Publications in Zoology, 67(1) : 1–124, plates 1–8, 40 figs., 3 maps.

Variation in rainbow trout was studied in specimens from Mexico and California, the following meristic characters being used: vertebrae, scales, branchiostegal rays, gill rakers, fin rays. Linear characters were also used. Bases for separating various populations of rainbow were found. In a discussion of the cutthroat series, the authors point out the recognition features of the Montana, coast, Yellowstone, Utah, Lahontan, and Independence Lake cutthroats and for rainbow-cutthroat hybrids and steelhead-cutthroat hybrids. The taxonomic status of the cutthroat as possibly being a subspecies of the rainbow is suggested.

OIEN, WAINE E.

1957. A prelogging inventory of four trout streams in Northern Idaho. Master of science thesis, University of Idaho. 92 pp.

In this study of the effects of logging on trout streams an assessment of streams was made before logging. Coastal cutthroat were native to the streams. Lengths and weights of various ages of cutthroat were determined in four streams in 1955 and 1956, prior to logging. Coefficients of condition ranged from 1.48 to 1.76.

OLIVE, JOHN R.

1953. A bibliography of the limnology and fishery biology of Colorado. Colorado Game and Fish Department (mimeographed). 34 pp.

Contains references to many reports and papers on cutthroat in Colorado.

PARISOT, THOMAS J., WILLIAM T. YASUTAKE, and VERNON BRESSLER.

1963. A new geographic and host record for infectious pancreatic necrosis. Transactions of the American Fisheries Society, 92(1) : 63–66.

The occurrence of infectious pancreatic necrosis in rainbow trout (*Salmo gairdneri*), brook trout (*Salvelinus fontinalis*), and cutthroat trout (*Salmo clarki*) has been experimentally authenticated for the first time in the western United States. The cutthroat trout represents a new host. Brook trout fin tissue culture inoculated with bacteria-free filtrate from the diseased fish tissue showed marked degenerative changes after 24 hours. Chinook salmon (*Oncorhynchus tshawytscha*), kokanee (*O. nerka*), and silver salmon (*O. kisutch*) were not susceptible to the virus when inoculated. Histologically extensive pancreatic necrosis was observed in the original and experimental materials, but striated muscle hyalinization was detected only in the original material.

PAUTZKE, CLARENCE F.

1938. Studies on the effect of coal washings on steelhead and cutthroat trout. Transactions of the American Fisheries Society, 67: 232–233.

Effects of coal washings in Cedar River in Washington were measured on steelhead and cutthroat. Cutthroat, 1 to 2 inches, died in 30 minutes, while the control was unaffected. Steelhead, 6 inches long, died in 1½ hours.

PECHACEK, LOUIS S.

1950. Harvest of wild and stocked fish from the Logan River drainage. Master's thesis, Utah State Agricultural College. 103 pp.

In this study of harvest of cutthroat, brook, brown, and rainbow trouts and mountain whitefish in Logan River in 1949, it was determined that cutthroat comprised 31.5 percent of the catch, rainbows 34 percent (25.5 percent were marked rainbows).

1956. The effects of tags on the rate of growth and condition of several species of coldwater fish in Wyoming. Progressive Fish-Culturist, 18(2) : 120–125.

Cutthroat and other salmonids in Wyoming were tagged with jaw and opercular tags. Loss in growth and in condition factor was exhibited after periods of from 2 months to 2 years.

PLATTS, WILLIAM S.

1957. The cutthroat trout. Utah Fish and Game Magazine, 13(10) : 4, 10.

This account of the cutthroat in Utah describes history and range of Utah cutthroat and Colorado cutthroat. Management of cutthroat in Utah is discussed in relation to environmental changes. Habitat, life history, spawning habits, and development of immature cutthroat are treated. Future for this fish in Utah is felt to be poor.

1958. Age and growth of the cutthroat trout in Strawberry Reservoir, Utah. Proceedings of the Utah Academy of Science, Arts, and Letters, 35: 101–103.

The body-scale relation of cutthroat in Strawberry Reservoir is calculated at $L = -16.7 + 2.47\ Sc$ and for tributary stream fish at $L = -1.4 + 1.88\ Sc$. Calculated lengths show reservoir cutthroat to average 413 mm. at the fourth annulus; tributary fish average 189 mm. at the fourth annulus. The maximum age found in the reservoir is 10 years. Tributary fish were found up to 4 years of age. Condition factors became smaller up to 3 years of age and increased beyond that age.

1959a. Homing, movements and mortality of wild cutthroat trout (*Salmo clarki* Richardson) spawned artificially. Progressive Fish-Culturist, 21(1): 36–38.

Cutthroat trout spawners with a strong drive to complete the spawning act were observed to travel the length of the 6-mile reservoir. Many marked spawners traveled 7 miles upstream, where migration was stopped by beaver dams. The high percentage of returns to the home stream gave supporting evidence of a homing instinct of cutthroat trout in an adfluvial environment.

The data indicate that mortality of both naturally and artificially spawned wild cutthroat trout is very high. Once it has completed spawning, a cutthroat trout in this type of environment is of little value to the future replenishment and should be made available to the creel.

1959b. Food habits of the cutthroat trout in Strawberry Reservoir, Utah. Proceedings of the Utah Academy of Science, Arts, and Letters, 36: 119–121.

In cutthroat stomachs from Strawberry Reservoir and its tributaries, chironomid pupae were the most important food in the reservoir, and *Daphnia* was important to the smaller cutthroat. In the tributaries caddis larvae and snails were the most abundant items. Fish were not prominent in the diet. Cutthroat fry and fingerlings fed on chironomid larvae and mayfly nymphs.

POST, GEORGE

1959. Nitrofuran compounds for furunculosis of trout, with special emphasis on Furoxone. Progressive Fish-Culturist, 21(1): 30–33.

Six nitrofurans were tested against *Aeromonas salmonicida in vitro*, with Furoxone giving the greatest inhibition. Furoxone was then tested against *Aeromonas salmonicida* in brook and cutthroat trout by addition to the diet, at 100 milligrams per kilo of fish per day. The furunculosis was controlled.

1962. Furazolidone (nf–180) for control of furunculosis in trout. Progressive Fish-Culturist, 24(4): 182–184.

Cutthroat and rainbow trout were used in experiments on the control of furunculosis. It is recommended that furazolidone at 25 mg. per kilogram of fish be used for at least 20 days for control. Roccal can also be used to disinfect cement ponds and raceways.

PROGRESSIVE FISH-CULTURIST.

1952. Marking cutthroat trout with Trypan Blue. Vol. 14 (No. 1), p. 9.

Trypan Blue gave prominent marks in fins and other parts of cutthroat trout at the Alsea Trout Hatchery in Oregon, but the dye failed to stay in the tissues for 3 weeks.

PURKETT, CHARLES A., Jr.

1951. Growth rate of trout in relation to elevation and temperature. Transactions of the American Fisheries Society, 80: 251–259.

A study of 758 trout from West Gallatin River indicated that growth was greater at lower elevations where water was warmer. Rainbow trout from upper and lower sections of West Gallatin River showed following differences in length at end of each year: 0.3 inch first year, 1.3 inches second year, 2.1 inches third year, 2.5 inches fourth year, and 4.0 inches fifth year. Growth rates of cutthroat and hybrid trout showed similar trend. Average summer difference in water temperature (early morning), between upper and lower stations of West Gallatin River, was 9.6° F. Condition factors did not show significant variations between sections.

Total of 184 trout from Bridger-Spring Creek did not show significant differences in growth rate. Water temperatures in stream did not vary greatly.

QADRI, S. U.

1959. Some morphological differences between the subspecies of cutthroat trout. *Salmo clarkii clarkii* and *Salmo clarkii lewisi*, in British Columbia. Journal of the Fisheries Research Board of Canada, 16(6): 903–922.

Coastal cutthroat trout, *Salmo c. clarkii* Richardson, occupy most lakes and streams of coastal British Columbia, including the adjacent islands. Yellowstone cutthroat, *Salmo c. lewisi* (Girard), occupy southeastern British Columbia; their range is separated from

that of the coastal subspecies by a zone lacking cutthroat trout. A map showing all natural distribution records in the Province is presented. From 60 to 146 specimens were examined for distinguishing characters. Spots below the lateral line are more numerous towards the anterior end in *Salmo c. clarkii*, but more numerous toward the posterior end in *Salmo c. lewisi*. A plot of spot number in selected areas of the body provides almost complete separation of individuals of the two subspecies. Significant differences also occur in certain scale counts and in body and peduncle depth, although these characters overlap considerably between the subspecies. No difference was found in vertebral count.

RASMUSSEN, DELBERT H.

1956. A creel census and fisherman expenditure study on Snake River, Wyoming. Wyoming Game and Fish Commission, Fisheries Technical Report No. 4. 26 pp.

A 40-mile section of Snake River was censused in 1955. Snake River cutthroat comprised 92 percent of catch and averaged 11 inches in length. Length frequencies and length-weight relations are given. Catch per hour for bank fishermen was 0.74, for boat fishermen 0.64.

1960. Preventing a winterkill by use of a compressed-air system. Progressive Fish-Culturist, 22(4): 185–187.

Cutthroat trout (*S. c. lewisi*) in Soda Lake in the Snake River drainage in northwestern Wyoming sustained a winterkill in 1957–58. Since the population was needed for brood stock and angling purposes, compressed air was used to remove ice cover from the lake. Pumping of air began in November 1958 and lasted 162 days. Eggtaking in May 1959 was successful.

RASMUSSEN, D. I.

1941. Beaver-trout relationship in the Rocky Mountain region. Transactions of the Fifth North American Wildlife Conference. pp. 256–263.

Area in Wasatch National Forest containing 620 miles of stream inhabited by cutthroat, almost 200 miles of which was stocked with beavers, was studied to learn effects beavers had on cutthroat. Physical and chemical measurements and bottom fauna counts suggested that beaver did not produce adverse effects on trout. Beaver dams obstructed trout movement to some extent.

RAYNER, H. J.

1947. Trout fishery in Oregon. Progressive Fish-Culturist, 9(3): 130–132.

In this description of Oregon's trout fishery, cutthroat play an important role. Anadromous and resident cutthroat west of the Cascades are discussed and Yellowstone cutthroat introductions are described.

REED, ROGER J.

1963. Results of preliminary watershed surveys conducted in 1961 and 1962 for studies on effects of forest insecticide spray on salmon streams in southeastern Alaska. Bureau of Commercial Fisheries, Manuscript Report 63–1. 12 pp.

In this study of DDT in streams prior to spraying for forest insect control, data were collected on stomach contents and on DDT residues of salmonids. Cutthroat stomach contents are reported. DDT and DDE were found in cutthroat from all four streams sampled.

RICH, WILLIS H.

1924. Destruction of trout by pelicans in Yellowstone National Park. Report of the U.S. Commissioner of Fisheries for 1923, App. 7. pp. 1–27.

In 1922, U.S. Bureau of Fisheries began investigation of pelicans in Yellowstone National Park, with Dr. H. B. Ward doing the work. He found that breeding period of white pelican in the park is synchronized with that of cutthroat trout. The pelican colony was 500–600 adults, and produced 200 young. Each pelican ate about 350 fish in the season. Control of pelicans by destruction of eggs was recommended.

RICKER, W. E.

1941. The consumption of young sockeye salmon by predaceous fish. Journal of the Fisheries Research Board of Canada, 5(3): 293–313.

Among the fish and insects which constitute the principal food of the larger predaceous fish of Cultus Lake, British Columbia, young sockeye salmon (*Oncorhynchus nerka*) are of outstanding importance. In general, the consumption of sockeye is proportional to their abundance, though it may be in some cases modified in the direction of less than proportional predation upon the smaller populations. Consumption of alternative foods increases greatly in years when sockeye are scarce.

Cutthroat trout prey on young sockeye throughout the year, from the time they rise from their nests until they leave the lake a year later. Consumption of sockeye is greatest in May and early June, just after emergence. Cutthroat were the most important predator in Cultus Lake in 1940.

Robertson, O. H.

1947. An ecological study of two high mountain trout lakes in the Wind River Range, Wyoming. Ecology, 28(2) : 87–112.

This study sought to explain difference in sizes of cutthroat trout from two adjacent, similar lakes. Life spans were about equal, but growth was faster in one lake. This lake had a better food supply, and its trout had food volumes in their stomachs thrice those of the smaller trout. No differences between the lakes were found in plankton counts, temperature, pH, O_2, dissolved Ca, or HCO_3. The richer lake had higher concentrations of phosphates, iodine, and organic matter.

Rounsefell, George A.

1957. Fecundity of North American Salmonidae. U.S. Fish and Wildlife Service Fishery Bulletin, No. 122, Vol. 57, pp. 451–468.

Measurements of egg diameters of cutthroat trout are compared with those of other salmonids. Measurements on number of eggs per female are given in relation to size of female, as well as other measures of egg size.

1962. Relationships among North American Salmonidae. Fish and Wildlife Service Fishery Bulletin 209, Vol. 62, pp. 235–270.

The relationships among species and genera of North American Salmonidae are assessed from published data on hybridization, coloration, and other attributes. The genus *Salmo* shows the greatest intrageneric variation. Phylogenetically, *Salmo gairdneri* is as close to the species of *Oncorhynchus* as to *Salmo salar;* and *Salmo trutta,* at the other extreme, is about midway between *S. salar* and the species of *Salvelinus.* The genus *Salvelinus* is a closely knit group. Of its species, *Salvelinus marstoni* shows the closest affiliation with *Salmo.*

Published data are scanty for several species, and the methods of taking and recording data vary so widely that comparison of data taken by different investigators is hazardous.

For cutthroat trout, data are presented on hybridization, coloration, anadromy, numbers of branchiostegal rays, numbers of pyloric caecae, counts of fin rays, numbers of vertebrae, numbers of gill rakers, and numbers of scale rows. Figures are also given for fecundity and weight of fish, and the distribution of the species is given in relation to temperature.

Rucker, R. R., and K. Hodgeboom.

1953. Observations on gas-bubble disease of fish. Progressive Fish-Culturist, 15(1) : 24–26.

Cutthroat and rainbow trout in a Washington hatchery were troubled with gas-bubble disease due to an excess of nitrogen in the water. When aeration lowered the nitrogen content to 110 percent of saturation the fish showed no evidence of gas-bubble disease.

Rucker, R. R., J. F. Conrad, and C. W. Dickeson.

1960. Ovarian fluid: Its role role in fertilization. Progressive Fish-Culturist, 22(2) : 77–78.

This study presents evidence that sperm from cutthroat trout and silver salmon were activated by ovarian fluid, that the ovarian fluid prolonged the viability of the sperm, and that eggs can be fertilized in ovarian fluid without the addition of water.

Rucker, R. R., W. T. Yasutake, and H. Wolf.

1961. Trout hepatoma—a preliminary report. Progressive Fish-Culturist, 34(1) : 3–7.

Hepatoma in trout is discussed and characterized as it occurs in rainbow and cutthroat.

Schneider, Phillip W., and Francis P. Griffiths.

1943. Production of trout in a small artificial pond in western Oregon. Journal of Wildlife Management, 7(2) : 148–154.

Stocking of trout in Cronemiller Pond resulted in no reliable survival data on rainbows and brown trout. Later stocking of cutthroat fingerlings with wild rainbow fry resulted in survival of 71.3 percent after 14.5 months for the cutthroat. Production was 46.5 pounds per surface acre.

Schoenthal, Norman D.

1963. Some effects of DDT on cold-water fish and fish-food organisms. Federal Aid to Fish Restoration Project Completion Report, Project Nos. F–21–R–4, Helena, Mont. (mimeographed). 47 pp.

Bioassays were conducted on six species of cold water fish, including wild cutthroat trout, to determine mortality rates under different concentrations of DDT and varying physical, chemical, and biological conditions. A total of 6,000 fish was tested. The mortality of rainbow trout varied little at DDT concentrations between 0.5 and 10 p.p.m. Mortality increased as the size of the fish decreased. Hatchery rainbow trout had a higher mortality than wild rainbow trout. Mortality increased as the temperature increased but decreased when the turbidity and alkalinity increased. Mortality was increased by feeding trout aquatic insects treated with DDT. A comparison of six species of fish tested in 1 p.p.m. DDT showed the longnose sucker had a mortality of 94 percent while the salmonids had less than 10 percent. Delayed mortality

occurred in all species of fish treated in DDT throughout a 6-month observation period.

Observations were made on fish and fish-food organisms in a test stream 8 months before, during, and 19 months after treatment with DDT which was applied at one point in the stream at 0.4 p.p.m. Immature aquatic insect populations were reduced 99 percent following application and required 18 months to regain pretreatment numbers. Immature aquatic insect populations below 0.75 mile downstream from the point of application regained 15 percent of the pretreatment numbers 11 months after application. The diet of trout in this downstream area changed from immature aquatic insects to adult insects and aquatic vegetation. Trout populations in the first mile of treated water showed no significant change 1 year following treatment but below this point showed a 73-percent reduction.

SCHULTZ, LEONARD P.

1934. Species of salmon and trout in the northwestern United States. Proceedings of the Fifth Pacific Science Congress of the Pacific Science Association (Can.), 5: 3777–3782.

Contains brief discussion and keys, in which cutthroat is included.

1936. Keys to the fishes of Washington, Oregon, and closely adjoining regions. University of Washington Publications in Biology, 2(4) : 103–228.

Contains keys to cutthroats.

1941. Fishes of Glacier National Park, Mont. U.S. Department of the Interior, Fish and Wildlife Service, Conservation Bulletin 22. 42 pp.

Has an account of distribution, keys to species, abundance, appearance, and habits of fishes of Glacier Park. Two forms of cutthroat, *S. c. lewisi* and *S. c. clarkii*, are treated. Detailed distributional tables for park waters and fishes are presented.

SIMON, JAMES R.

1935. A new species of nematode, ***Bulbodacnitis scotti***, from the trout, *Salmo lewisi* (Girard). University of Wyoming Publications, 2(2) : 11–15.

Contains description of new nematode from intestine and other viscera of *Salmo lewisi* in Yellowstone Lake.

1939, 1953, 1962. Yellowstone fishes. Yellowstone Library and Museum Association. 49 pp.

Fish-cultural practices for cutthroat in Yellowstone, parasites of cutthroat in the park, and natural predators on cutthroat are discussed. Description of Yellowstone cutthroat is given with notes on habits and distribution in Yellowstone.

1946. Wyoming fishes. Wyoming Game and Fish Department, Bulletin No. 4. 129 pp.

Keys to identification of cutthroat are given, and sections on *S. clarki lewisi*, cutthroat-rainbow hybrids, *S. c. pleuriticus*, and *S. c. stomias* cover distribution in Wyoming, biology, and fishing. Illustrations of cutthroat and hybrids are included.

SLATER, JOHN V.

1961. Comparative accumulation of radioactive zinc in young rainbow, cutthroat, and brook trout. Copeia, No. 2. pp. 158–161.

An analysis of the comparative accumulation of radioactive zinc in young rainbow, cutthroat, and brook trout reveals that the greatest rate of accumulation of this isotope is in the brook trout, the least in the rainbows. A study on the localization of this element showed that in the rainbow trout and the cutthroat, the gill filaments accumulated the greatest amount of isotope while in both instances the least amount was discovered in the ventral body wall. Ion uptake in the cutthroat gill was greater than in that of the rainbow with this latter species being found to accumulate only about one-half that discovered in the cutthroat gill.

The rate of accumulation of zinc in the rainbow trout was only 75 percent that found in the brooks. The final total concentration of the ion in 48 hours was found to be greatest in the brook trout, with about 95 percent of this concentration being found in the cutthroats and about 70 percent of the highest concentration seen in the rainbows.

SMITH, HUGH M., and WILLIAM C. KENDALL.

1921. Fishes of the Yellowstone National Park. Report of the U.S. Commissioner of Fisheries for 1921, App. 3. pp. 1–30.

Cutthroat is listed as one of indigenous species in Yellowstone. Hatchery operations with cutthroat are described. Section on cutthroat gives detailed distribution within Yellowstone Park, discusses fishing, and gives something on tapeworm parasite of fish in these waters.

SMITH, OSGOOD R.

1941. The spawning habits of cutthroat and eastern brook trouts. Journal of Wildlife Management, 5(4) : 461–471.

Spawning cutthroat were studied in Convict Creek, Calif., and observations made on distribution of fish and spawning periods, numbers of eggs retained by females, spawning activities, night spawning, egg predation, guarding completed redd, and damage to eggs by overlapping of redds. Contains excellent photographs of spawning.

1947. Returns from natural spawning of cutthroat trout and eastern brook trout. Transactions of the American Fisheries Society, 74 : 281–296.

Experiments were conducted over three seasons to determine number of fingerlings or fry produced by natural spawning of cutthroat (*Salmo clarkii henshawi*) and eastern brook trout (*Salvelinus fontinalis*). Returns were secured by inducing wild trout to spawn in controlled and screened section of stream. Resulting fingerlings were collected by diverting water and pumping pools dry.

Cutthroat spawned in May and fingerlings were recovered in September each year.

During first summer, horizontal screens, acting as fish traps, were used below cutthroat spawning areas so that some fry were trapped soon after emerging from gravel. Other fry remained in the section and were recovered in September. Minimum survival was about 17 percent of estimated number of eggs laid. Data were obtained on fry migration and growth of fry spawned at same time.

Vertical screens were used in all other experiments so that all survivors were collected in September. Survivals of cutthroat ranged from nothing to about 6 percent of estimated number of eggs in four trials, each trial section containing one to three female spawning trout.

SMITH, S. B.

1955. The relation between scale diameter and body length of Kamloops trout, *Salmo gairdneri kamloops*. Journal of the Fisheries Research Board of Canada, 12 (5) : 742–753.

The scale-body relation for Kamloops trout is very close to direct proportion for fish larger than 4.5 cm. fork length. Observations on steelhead trout, *Salmo gairdneri gairdneri*, and on coastal cutthroat trout, *Salmo clarki clarki*, suggest that an isometric scale-body relation may exist among those species also. McMynn and Vernon have shown relation in cutthroat to be linear with a log-log regression-line slope of 1.019, compared with 1.024 for Kamloops and 1.010 for steelhead.

SNYDER, GEORGE R., and HOWARD A. TANNER.

1960. Cutthroat trout reproduction in the inlets to Trappers Lake. Colorado Department of Game and Fish, Technical Bulletin No. 7. pp. 1–86.

An evaluation of the reproductive potential of the cutthroat trout which spawn in the inlet streams to Trappers Lake in Colorado was made from June to September, 1958 and 1959. Information was recorded for times of spawning runs, numbers of spawners, sizes of spawners, age distribution of spawners, sex ratios in spawning runs, numbers of spawners returning to spawn the second year, fecundity, physical condition of spawning areas, times of fry migration, numbers of fry migrating, and survival of hatching eggs.

SNYDER, J. O.

1917. Fishes of the Lahontan system of Nevada and Northeastern California. Bulletin of the U.S. Bureau of Fisheries, 35 : 31–86.

In this account of all fishes in this basin, *Salmo henshawi* is given a long treatment. Details of color patterns from different drainages are given, as well as counts of spots on body areas. Very detailed measurements of specimens from different waters, of both sexes, and of different size groups are shown in tables. Migrations of cutthroat are discussed. The famous large cutthroat of Pyramid Lake are described. Methods of fishing and types of gear used are discussed.

1940. The trouts of California. California Fish and Game, 26 (2) : 96–134.

Descriptions of morphology, distributions, and biologies and life histories of *Salmo clarki*, *Salmo henshawi*, *Salmo seleniris*, and *Salmo pleuriticus* are given in this treatment of trouts in California.

SUCKLEY, GEORGE.

1874. On the North American species of salmon and trout. Report of the U.S. Commissioner of Fisheries for 1872–73, pp. 91–160.

This catalog of North American Salmonidae classifies the genus *Salmo*, with anadromous salmons and trouts separated from nonanadromous species. Each species is individually treated with discussion of nomenclature, morphological characteristics, colors, diagnostic features, habitat, life-history notes, and taxonomic relations. Cutthroats treated are *Salmo tsuppitch* Richardson, *Salmo clarkii* Richardson, *Salmo virginalis* Girard, *Salmo lewisi* Girard, *Salmo stellatus* Girard, *Salmo gibbsii* Suckley, and *Salmo brevicauda* Suckley.

SUMNER, FRANCIS H.

1940. The decline of the Pyramid Lake fishery. Transactions of the American Fisheries Society, 69 : 216–224.

History of Pyramid Lake (Nevada) cutthroat trout (*Salmo henshawi* Gill and Jordan)

fishery, conducted by reservation Indians, shows a rapid decline in catch within last few years. While the Indian is blamed for overfishing, the white man's demand for trout, the dams and diversions that he built along the Truckee River where the trout have spawned, the pollutants that he has dumped into the river, the spawn that he has taken, the competing species of fish that he has introduced, and principally the large amounts of water that he has diverted to another watershed, have led to a condition which has prevented the trout from reproducing its kind. Revival of the fishery is made doubtful by an agreement concerning use of Truckee River's flow, expense of hatching and rearing a stock of Pyramid Lake trout (not to mention questionable availability of its eggs), and inadequate regulation of the fishery. The Pyramid Lake cutthroat trout fishery appears to be doomed.

1948. The coast cutthroat trout. Oregon State Game Commission Bulletin, 3(12) : 1, 6–8.

Study on a coastal stream, Sand Creek, was begun in 1946, with upstream-downstream traps. "Blue-back" trout (salmon-trout, harvest trout) appear in coastal streams in October in many color phases. Upstream mortality was around two-thirds. Sand Creek cutthroat spend 1 to 5 years in the stream before migrating to the ocean, most doing so at 3 years of age. In the 1946 spawning run, 19.2 percent had spawned once before, 7.0 percent twice, and 1.5 percent three times. Some may spawn without ever having gone to the ocean.

1953. Migrations of salmonids in Sand Creek, Oregon. Transactions of the American Fisheries Society, 82 : 139–150.

Upstream and downstream fish traps were maintained on Sand Creek, a small Oregon trout stream, during four upstream and three downstream fish runs (1946–49), and a smaller trap was operated on a tributary during part of 1947. Two trouts, *Salmo clarki clarki* and *Salmo gairdneri gairdneri*, and two salmons, *Oncorhynchus kisutch* and *Oncorhynchus keta*, made spawning runs in fall and winter ; downstream migrations of fry and fingerlings occurred in the spring.

Water temperatures and information on occurrence and magnitude of freshlets were taken. Records on fish trapped included numbers of salmonids, lengths, weights, sex ratios, loss in weight after spawning, and survival rates.

Cutthroat upstream migrants ranged from 4.0 to 10.7 inches in fork length for one size group and from 11.0 to 18.6 inches in another size group. Sex ratios of sea-run cutthroat showed predominance of females (1.00 male to 1.69 female). Weights of migrating cutthroat ranged from 0.50 to 2.94 pounds. Fork lengths of downstream migrants were from 2.7 to 10.9 inches.

Survival of cutthroat spawners averaged 32 percent. Weight loss of spawners was about one-third.

1962. Migration and growth of the coastal cutthroat trout in Tillamook County, Oregon. Transactions of the American Fisheries Society, 91(1) : 77–83.

Coastal cutthroat trout (*Salmo clarki clarki* Richardson) were studied at Sand Creek, Tillamook County, Oreg. (1946–49). Upstream runs occurred mainly in fall and early winter months and downstream runs in the spring. Upstream migrant sea-run cutthroat averaged 13.6 inches, while upstream nonsea trout averaged 6.0 inches, fork length. Downstream trout, mainly initial migrants, averaged 5.7 inches long. Of summer-resident trout, 8.7 percent were of legal angling length. Scales of sea-run trout gave ages of 2 to 10 years, with different combinations of stream and sea life. Most sea-run trout spawned after one season in the sea and a third of the run had spawned one to three times. Growth was back calculated from scale reading. Survival figures are given.

Tanner, V. M.

1936. A study of the fishes of Utah. Proceedings of the Utah Academy of Science, Arts, and Letters, 13 : 155–184.

This treatment of fishes of Utah has keys to several forms of cutthroat, and a discussion of findings of some early explorers with regard to distribution and abundance of cutthroat in Utah waters.

Tanner, V. M., and Sheldon P. Hayes.

1933. The genus Salmo in Utah. Proceedings of the Utah Academy of Science, Arts, and Letters, 10 : 163–164.

Two cutthroats, *S. utah* Suckley and *S. pleuriticus* Cope, are in Utah. Yellowstone and Pyramid Lake cutthroats have been introduced and have mixed with native forms. Suckley, Goode, Jordan and Evermann, and Cope are quoted with regard to cutthroats on which they worked.

Thompson, R. B.

1959. Tricaine Methanesulfonate (M.S. 222) in transport of cutthroat trout. Progressive Fish-Culturist, 21(2) : 96.

Tricaine methanesulfonate was used to reduce the water-to-fish ratio for planting of cutthroat trout near Seattle, Wash. The best method was to use 500 ml. of a 1 : 40,000 concentration of the agent in 1-quart plastic food containers. Thus, 500 cutthroat, 3,000 to the pound, were put into each container, the lid sealed, and the container put into an iced picnic bag. Three hours later, only 19 out of 2,000 fish were considered to be dead.

TRYON, C. A. Jr.

1943. The effect of covering hatchery troughs on the growth of cutthroat trout (*Salmo clarkii*). Transactions of the American Fisheries Society, 72 : 145–149.

An experiment was designed and carried out to test effect of covered troughs on growth rate of cutthroat trout at Bozeman, Mont. Analysis of data showed trout in open troughs to have significantly greater growth rate than those in covered troughs. Growth rate in open troughs was greater by 13.8 percent.

TUNISON, A. V., and C. M. MCCAY.

1933. The nutritional requirements of brook trout. Transactions of the American Fisheries Society, 63 : 167–177.

In this study on nutrition of trout a group of cutthroat trout became infested with *Octomitis salmonis*. Antiseptics were fed in liver. Control of moving forms was achieved with mercurous chloride, 0.2 percent, Hexamethylenamine, 0.5 percent, and Naphthol (beta), 0.5 percent, and partial control with carbon tetrachloride, 0.2 percent.

TURNER, C. L.

1946. A case of hermaphroditism in the cutthroat trout. Chicago Academy of Sciences Natural History, Miscellanea No. 1, pp. 1–2.

A female cutthroat (*Salmo clarkii*) from Yellowstone Lake was examined by the author. Ovaries were normal. At the anterior end of the right ovary was a flattened oval mass, which was examined histologically. This was a small testis, with mature spermatozoa and nests of spermatogonia.

Hermaphroditism as a normal situation in other fishes is discussed.

UNITED STATES COMMISSION OF FISH AND FISHERIES.

1900. A manual of fish culture. From Report of the U.S. Commissioner of Fisheries for 1897. 340 pp.

This manual deals with all aspects of culture of fishes raised in the United States. A small section is devoted to cutthroats.

VERNON, E. H., and R. G. MCMYNN.

957. Scale characteristics of yearling coastal cutthroat and steelhead trout. Journal of the Fisheries Research Board of Canada, 14(2) : 203–212.

Some scale characteristics of 88 yearling coastal cutthroat trout (*Salmo clarki clarki*) and 96 yearling steelhead (*Salmo gairdneri*) were compared in some detail. Although the number of diagonal scale rows above the lateral line was significantly higher in cutthroat, counts of the two species overlap. Correlation of scale circuli counts with scale diameter was 0.94 for steelhead and 0.90 for cutthroat. At any given scale size, cutthroat scales had 8 percent more circuli than steelhead scales. Measurements of lateral and longitudinal diameters and anterior radii of scales show that the anterior portions of cutthroat scales tend to be longer and narrower than those of steelhead.

Regression analyses of each scale measurement on fork length indicated that, for any given fork length, cutthroat scales are smaller than those of steelhead, the greatest difference being apparent when anterior radii are compared. Growth of scales in a dorsoventral plane approximates isauxesis in relation to fork length, but scale growth in a longitudinal plane tends toward positive heterauxesis which is particularly pronounced in the anterior portions of scales.

Multiple regression analyses of scale diameter, scale row count, and fork length indicated that the smaller size of cutthroat scales is not balanced by proportionate increase in their number. When scale diameter is adjusted for scale number and fork length, cutthroat scales are 15.6 percent smaller than those of steelhead, indicating that young cutthroat are covered by their scales with less overlap.

WALES, J. H.

1957. Trout of California. California Department of Fish and Game, Sacramento. 56 pp.

The coast cutthroat trout, the Piute cutthroat trout, and the Lahontan cutthroat trout are discussed with regard to distinguishing characteristics, distribution in California, and interesting facts. A figure of each subspecies is given.

WARD, H. B.

1923. The pelicans of Yellowstone Lake. Typewritten report to the U.S. Bureau of Fisheries. pp. 1–20.

Relations of the white pelican and the fish of Yellowstone Lake in 1922. Feeding habits of the pelicans were closely observed. Of the fish eaten, 98 percent were cutthroat trout.

It is estimated that 350,000 cutthroat were taken by pelicans in 1922.

WEISEL, GEORGE F.

1957. Fish guide for intermountain Montana. Montana State University Press, Missoula. 88 pp.

This contains a discussion of the differences between *Salmo clarki clarki* and *S. c. lewisi* in Montana. Some notes on biology and spawning habits are included.

WELSH, JAMES P.

1952. A population study of Yellowstone blackspotted trout (*Salmo clarkii lewisi* Girard). Ph. D. dissertation, Leland Stanford Junior University. pp. 1–180.

A study of cutthroat populations in Arnica Creek, tributary of Yellowstone Lake, Yellowstone National Park, was made in 1950 and 1951. Sex ratio in the 1951 runs was 90 males to 102 females. Length-weight regression of Arnica Creek resident trout was calculated at Log Y=3.002 Log X—5.063. Egg counts ranged from 745 to 1,495 per female. Times of spawning runs and their variation within the season are discussed at length.

WHITNEY, ARTHUR N., and JOHN C. SPINDLER.

1959. Effects of kraft paper wastes on a Montana stream. Transactions of the American Fisheries Society, 88(2) : 153.

A paper mill began operating in November 1957 on the Clark Fork River, discharging kraft process wastes into the river. Cutthroat and other fish were found dead for 25 miles downstream the following July, and pathological examination showed death to be by poisoning. Bottom fauna were seriously reduced at a station 2 miles downstream. Rainbows in live cars one-half mile below the effluent outlet all died in 30 hours.

WILDE, EARL.

1961. Cutthroat brood stock. Wyoming Wildlife, 25(5) : 25–27.

The decline of the cutthroat in the Rocky Mountains is discussed, and the development of a brood stock of Snake River cutthroat is described. Details of the culture of eggs and fry leading to the brood stock are given, and the care and use of the progeny are discussed. There is promise that selective breeding will result in earlier dates of maturity and improved brood stock.

WILLOUGHBY, HARVEY.

1951. Age and growth of the cutthroat trout, *Salmo clarkii lewisi*, in Yellowstone Lake. Yellowstone Nature Notes, Yellowstone Library and Museum Association, 25(3) : 29–32.

Scales were read from 523 cutthroat collected from Yellowstone Lake in 1949. Age distribution and growth rate were figured. Year class IV was best represented, followed by III, V, and VI.

WOLF, KEN.

1954. Progress report on blue-sac disease. Progressive Fish-Culturist, 16(2) : 51–59.

In this report on the history, symptoms, and course of blue-sac disease, the appearance of the disease in Yellowstone cutthroat is treated. Photographs of normal and diseased cutthroat are shown.

WOOD, E. M., and W. A. DUNN.

1948. Fact and fiction in spawntaking. Progressive Fish-Culturist, 10(2) : 67–72.

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1959. Observations on the movements and reproduction of the Cascade form of cutthroat trout. Master of science thesis, Oregon State College. 60 pp.

Cutthroat in Lookout Creek on the Willamette National Forest were studied in 1956 and 1957. Of the trout in the system, 95 percent were less than 4 years old. No downstream movement of adults was seen. Two up-

stream movements, one from October through January, and the other from March to June, took place. The spring movement was a reproductive migration. Fry moved downstream from June to August. Small tributaries may be insignificant as brood waters for Lookout Creek.

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Life history of red-throated trout, *Salmo virginalis*, in Utah Lake, treating spawning, feeding, and movements. Maximum length was 3 feet, maximum weight 15½ pounds.

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  Neave, 1936, 1943.
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  Cordone and Kelley, 1961.
  Griffin, 1938.
Sex ratio:
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  Bulkley, 1959.
  Hartman, 1956.
  Irving, 1953, 1956.
  Madsen, M., 1940.
  Snyder and Tanner, 1960.
  Sumner, 1953.
  Welsh, 1952.
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  Code, 1950.
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Shocking:
  Morris, 1950.
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  Bangham, 1951.
  Jordan, 1890.
  Rasmussen, D. H., 1956, 1960.
Soils:
  Benson, 1961.
Spawning:
  Ball and Cope, 1961.
  Calhoun, 1944a.
  Cope, 1957c.
  Cope and Yarrow, 1875.
  Cramer, 1940.
  Dimick and Merryfield, 1945.
  Drummond and Tanner, 1960.
  Dymond, 1932.
  Foster, 1933.
  Hazzard, 1932.
  Juday, 1907a.
  Leach, 1939.
  Mason, 1953.
  Neave, 1949.
  Platts, 1957.
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  Snyder and Tanner, 1960.
  Sumner, 1948.
  Weisel, 1957.
  Yarrow, 1874, 1875.
  Wood and Dunn, 1948.
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  Jordan, 1928.
Sperm:
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  Rucker, Conrad, and Dickeson, 1960.
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  Bjornn, 1957.
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  Gee, 1942.
  Madsen, 1936.
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  Schneider and Phillips, 1943.
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  Platts, 1958, 1959b.
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  Ball and Cope, 1961.
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  Drummond and Tanner, 1960.
Tricaine methanesulfonate:
  Thompson, R. B., 1959.
Trout, black:
  Jordan and Henshaw, 1879.
  Suckley, 1874.
Trout, Clark's:
  See Trout, red-throated.

- Trout, green-backed:
  - Bulkley, 1959.
  - Ellis, 1914.
  - Greene, 1937.
  - Jordan, 1891a.
  - Jordan and Evermann, 1902.
  - Land, 1913.
  - Simon, 1946.
- Trout, red-throated:
  - Bean, 1894a.
  - Cope and Yarrow, 1875.
  - Evermann, 1893.
  - Jordan, 1890, 1891a, 1891b, 1920.
  - Kendall, 1920.
  - Linton, 1891a, b, 1893.
  - Suckley, 1874.
  - Yarrow, 1874, 1875.
- Trout, Rio Grande:
  - Cope and Yarrow, 1875.
  - Ellis, 1914.
  - Greene, 1937.
  - Jordan, 1891a, 1920.
  - Land, 1913.
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  - See Trout, red-throated.
- Trout, Waha Lake:
  - Jordan, 1891a.
- Trout, yellow-finned:
  - Ellis, 1914.
  - Greene, 1937.
  - Jordan, 1891a.
  - Jordan and Evermann, 1902.
- Trypan Blue:
  - Progressive Fish-Culturist, 1952.
- Tumor:
  - Adami, 1908.
- Twin Lakes, Colo.:
  - Juday, 1907b.
- Two-Ocean Pass, Wyo.:
  - Daniels, 1952.
  - Jordan, 1928.
- Upper Blue Lake, Calif.:
  - Calhoun, 1944a,b.
- Upper Pecos River, N. Mex.:
  - Gee, 1941.
- Utah:
  - Cope, 1955.
  - Cope and Yarrow, 1875.
  - Fleener, 1952.
  - Foster, 1933.
  - Hatton, 1932.
  - Jordan, 1891a.
  - Laakso, 1955.
  - Lord, 1930.
  - McConnell, Clark, and Sigler, 1957.
  - Pechacek, 1950.
  - Platts, 1957, 1958, 1959b.
  - Tanner, 1936.
  - Tanner and Hayes, 1933.
  - Yarrow, 1874.
- Utah Lake:
  - Hatton, 1932.
  - Yarrow, 1874.
- Vertebrae:
  - Hartman, 1956.
  - Mottley, 1937.
  - Needham and Gard, 1959.
  - Qadri, 1959.
  - Rounsefell, 1962.
- Viability:
  - Hartman, 1956.
- Washington:
  - Chapman and Quistorff, 1938.
  - Pautzke, 1938.
  - Schultz, 1936.
  - Thompson, R. B., 1959.
- Water levels:
  - Benson, 1960.
  - Bulkley and Benson, 1962.
- Water quality:
  - Benson, 1961.
- Western United States:
  - DeWitt, 1954b.
  - Miller, 1950.
- Willamette River:
  - Dimick and Merryfield, 1945.
- Wind River Range, Wyoming:
  - Robertson, 1947.
- Winterkill:
  - Rasmussen, 1960.
- Wyoming (not including Yellowstone):
  - Beal, 1959.
  - Evermann, 1893.
  - Grasse, 1949.
  - Hazzard, 1932.
  - Jordan, 1928.
  - Pechacek, 1956.
  - Rasmussen, D. H., 1956.
  - Robertson, 1947.
  - Simon, 1946.
  - Wilde, 1961.
- Year-class:
  - Benson, 1960.
  - Bulkley, 1961.
  - Bulkley and Benson, 1962.
- Yellowstone Lake:
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  - Ball and Cope, 1961.
  - Bangham, 1951.
  - Benson, 1958, 1960, 1961.
  - Biesinger, 1961.
  - Brown and Bailey, 1952.
  - Bulkley, 1961, 1963.
  - Bulkley and Benson, 1962.

  - Cope, 1953a,b, 1956, 1957a,b,c, 1958b.
  - Hall, M. C., 1930.
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  - Kendall, 1915.
  - Laakso, 1956.
  - Laakso and Cope, 1956.
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  - Madsen, M., 1940.
  - Moore, Cope, and Beckwith, 1952.
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  - Wood and Dunn, 1948.
  - Woodbury, 1934.
- Yellowstone River :
  - Ball and Cope, 1961.
  - Cope, 1961.
- Zinc :
  - Slater, 1961.

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EFFECTS OF ACCUMULATED EXCRETORY PRODUCTS ON HATCHERY-REARED SALMONIDS

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RESEARCH REPORT 66

RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two Bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

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EFFECTS OF ACCUMULATED EXCRETORY PRODUCTS ON HATCHERY-REARED SALMONIDS

Roger E. Burrows
Fishery Research Biologist

RESEARCH REPORT 66

UNITED STATES DEPARTMENT OF THE INTERIOR
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FISH AND WILDLIFE SERVICE
Clarence F. Pautzke, *Commissioner*
BUREAU OF SPORT FISHERIES AND WILDLIFE
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ABSTRACT

In experiments at the Bureau's Salmon-Cultural Laboratory, to determine the excretory products of significance in rearing ponds and the possible effect of these products on fingerling chinook salmon (*Oncorhynchus tshawytscha*), the results demonstrate that ammonia and urea are the principal products. The relative amounts of these products vary with the concentration of fish and with their metabolic activity. Ammonia assumes dominance in an unfavorable environment. The pH, water temperature, and pond type affect the toxicity of ammonia solutions. Continuous exposure to levels of ammonia normally encountered in rearing ponds produced extensive hyperplasia of the gill epithelium in controlled experiments. In rearing ponds, prolonged but not continuous exposure in unionized ammonia has been demonstrated to result in reduction both in growth rates and in physical stamina. It is postulated that the gill hyperplasia resulting from continuous exposure to un-ionized ammonia is a precursor of bacterial gill disease.

EFFECTS OF ACCUMULATED EXCRETORY PRODUCTS ON HATCHERY-REARED SALMONIDS

By Roger E. Burrows, *Fishery Research Biologist*

Salmon-Cultural Laboratory, Bureau of Sport Fisheries and Wildlife
Longview, Washington

This is the second report on factors that limit production in rearing ponds. It is concerned principally with excretory products, their accumulation, and their effects on hatchery-reared salmonids.

The first report, by Burrows and Chenoweth (1955), was on investigations designed to explore the hydraulic and biological characteristics of several types of rearing ponds. Its principal purpose was to define the problem. The results emphasized the lack of information applicable to the measurement of environmental effects and the need for more intensive investigations into the limitation imposed by water quality, excretory products, water temperature, pond types, and the reuse of water.

The effect of excretory products was selected as the second study because it is not only a relatively unexplored field but also one of the most critical hatchery problems. The method of attack was first to define the significant excretory products in rearing ponds, and second to measure the effect of these products on the fish.

PRINCIPAL EXCRETORY PRODUCTS IN REARING PONDS

Salmon and trout rearing ponds vary in size and shape, but all are supplied continuously with water. The problem of measurement of the excretory products is complicated by this constant interchange. Most investigators have confined their experiments to aquariums which allow the excretory products to accumulate, thus leading to erroneous conclusions about the normal relations of certain products of excretion.

Ammonia nitrogen has been found to be one of the principal products of excretion. Because of the confusion of terminology in the literature, this term needs further definition. According to dictionary definition (Webster, 1951) ammonia itself is the gas NH_3, and ammonia nitrogen is nitrogen combined in the form of ammonia, NH_3, or ammonium, NH_4. The ammonium radical NH_4 combines with a number of elements, but as a product of excretion it is present as ammonium hydroxide which dissociates in a reversible reaction into ammonia, NH_3, and water. In some of the more recent literature (Wuhrmann and Woker, 1949; Downing and Merkins, 1955; Merkins and Downing, 1957; and Lloyd and Herbert, 1960) ammonia and ammonia nitrogen are used almost as synonyms, with the term ammonia referring to aqueous ammonia containing both the gas, NH_3, and ammonium hydroxide, NH_4OH. The ammonia gas, NH_3, is identified, specifically, as un-ionized ammonia. This terminology will be used in this report. Most of the earlier work considers ammonia in the same sense with the exception that the significance of the un-ionized form is not recognized.

Smith (1929) found that the principal end products of catabolism in carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) were ammonia and urea. Wood (1958) found that in three species of marine fishes 75 to 98 percent of the total nitrogen excreted consisted of ammonia and urea. Both Smith and Wood analyzed the water from aquariums in which fish had been held for 18 to 24 hours to determine the products of excretion. Both workers found that the amount of ammonia excreted was much greater than that of urea.

Our approach was different in that hourly samples of water from rearing pond effluents were analyzed over 24-hour periods to determine the significant products of excretion. Our problem, of course, was different in that we were interested primarily in how the products of excretion affected the fish in rearing ponds.

Analyses of the effluents were made for ammonia, urea, creatinine, and uric acid; ammonia by direct nesslerization (Ellis et al., 1946), urea by the Karr method (Karr, 1924), creatinine by the method of Folin and Wu (1919), and uric acid by the method of Folin (1933, 1934). Urea and ammonia were the major excretory products as these were the only products which appeared in measurable amounts. Contrary to both Smith's and Wood's results, either ammonia or urea could be the principal excretory product dependent either on the size and weight of fish in the pond or on the time of day of the analysis.

The effluent concentrations of ammonia and urea from several pond types were measured in a series of experiments. The results of one of these evaluations is shown in figures 1 and 2. In this experiment, two 4- by 40-foot raceway ponds were stocked with equal poundages of comparable chinook salmon (*Oncorhynchus tshawytscha*) fingerlings. The water inflow into one pond was half that of the other, 125 gallons per minute as compared with 250 gallons per minute. A similar difference in the ratio of fish weight to water inflow was maintained throughout a growing season as the total weight of fish increased. At intervals of approximately 6 weeks, the effluent waters were analyzed for both ammonia and urea at hourly intervals for 24 hours. The results of these analyses for the high inflow pond are shown in figure 1. With the low loading rate used in this pond, urea was the principal product of excretion throughout the season. At the higher loading rate, and an average water temperature of 52° F. (fig. 2), ammonia soon became the predominant excretory product. This dominance was demonstrated at a loading rate of 5.6 pounds of fish per gallon per minute of inflow. At higher metabolic rates, influenced both by fish size and by water temperature, ammonia dominance would be expected at a lower loading rate. At the end of August as shown in figure 1, ammonia dominance was approached in the raceway with the higher inflow, when the temperature reached 55° F. and the weight of fish per gallon per minute of inflow was 4.37 pounds.

The physiological processes involved in the suppression of urea excretion have not been explored. Certainly no reference to this phenomenon has occurred in the literature principally because the experi-

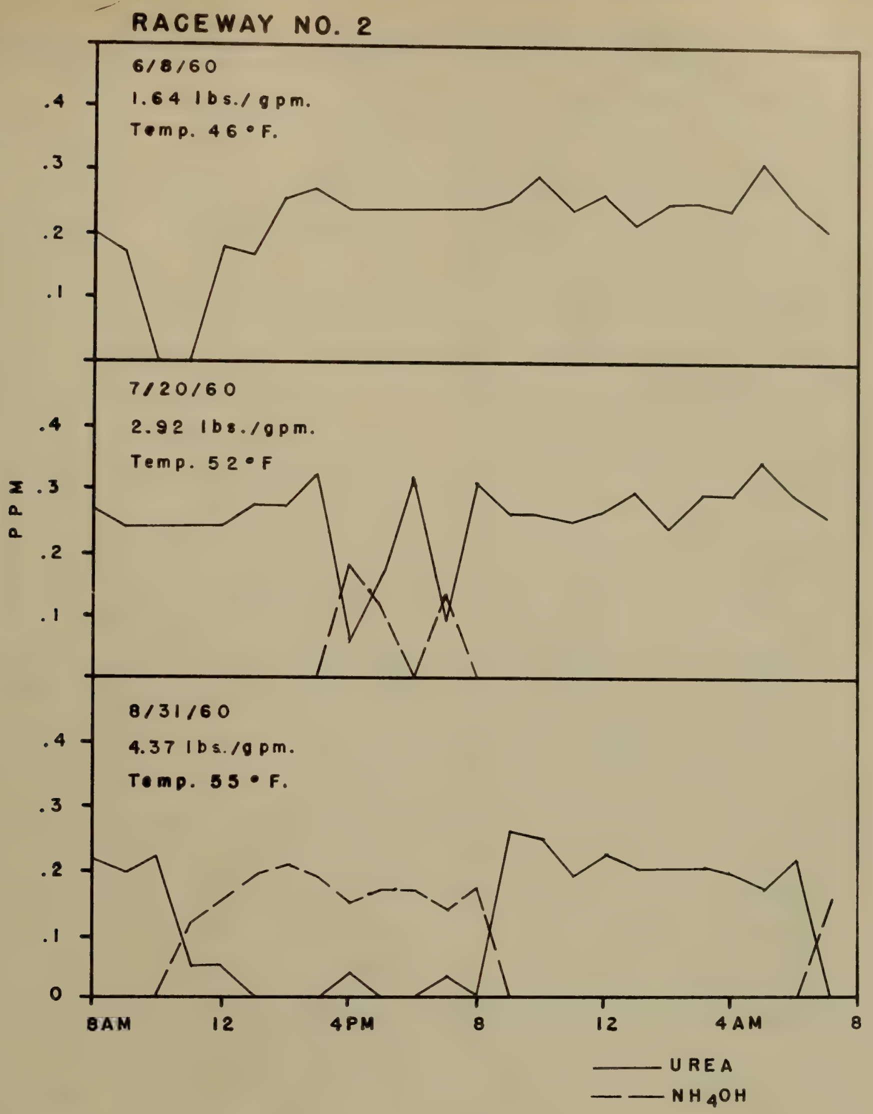

FIGURE 1.—Patterns of ammonia and urea excretion in a raceway pond with high inflow. Ammonia nitrogen expressed as NH_4.

mental procedures employed accumulated the excretory products in small quantities of water for comparatively long periods of from 18 to 24 hours with aeration but without water replenishment. The results of such conditions are similar to those encountered at the 8.16 pound loading rate shown in figure 2 in which ammonia predominated and urea was present in very small quantities.

Although figure 1 shows no ammonia excretion at the low loading rate of 1.64 pounds, such was probably not the case. The direct nesslerization method for the measurement of ammonia nitrogen is not accurate below 0.1 p.p.m. Thus, small quantities of ammonia could have been and probably were being excreted by the fish even though no ammonia was indicated by the measurements.

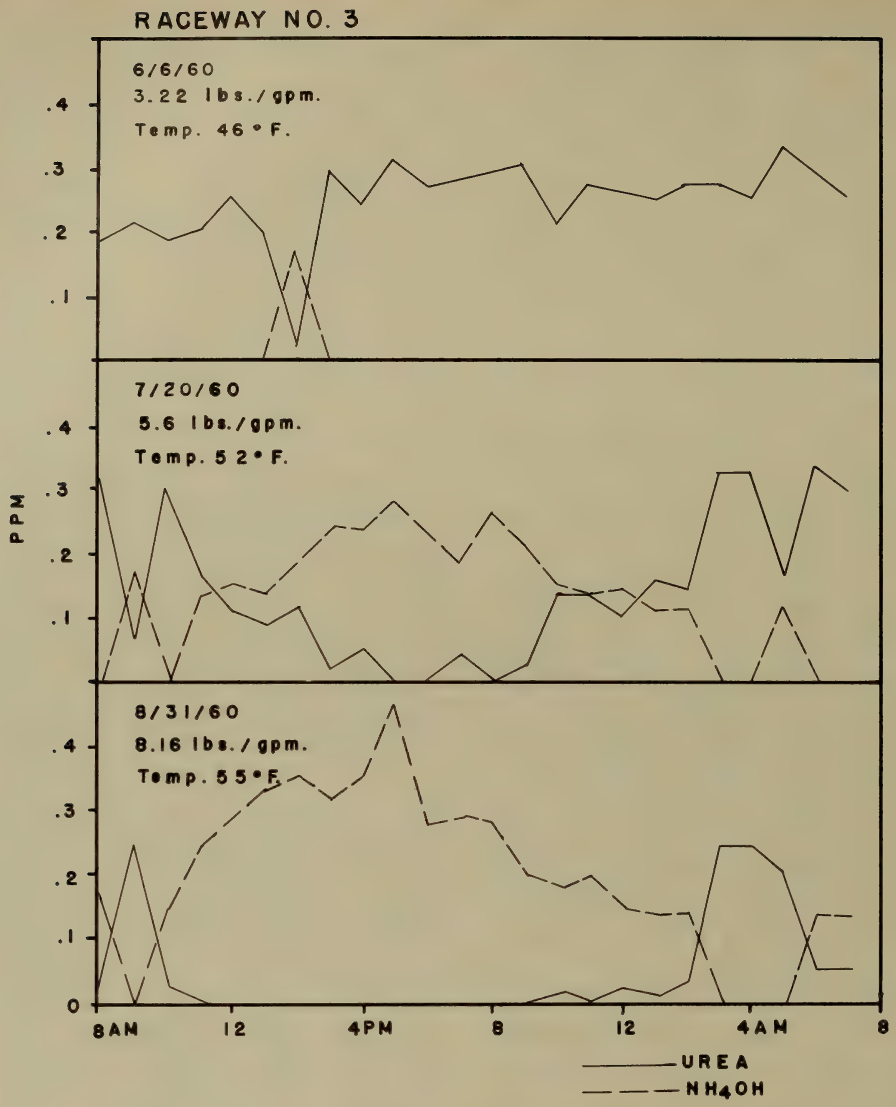

Figure 2.—Patterns of ammonia and urea excretion in a raceway pond with low inflow. Ammonia nitrogen expressed as NH_4.

EFFECTS OF EXCRETORY PRODUCTS ON THE FISH

Although various pond types studied over a 10-year period differed in their expulsion rates and therefore in the rate of removal of waste products from the pond water, the domination of ammonia was characteristic of high loading rates in all tests. Since fish mortality is also associated with very high loadings, ammonia was suspect as a toxin. In several experiments where overloads were associated with outbreaks of gill disease, ammonia was present continuously in the pond effluent and urea was either unmeasurable or present in but small quantities and for but short periods of time.

To measure the effect of ammonia under controlled conditions, two experiments were conducted for a period of 6 weeks, at water temperatures of 43° F. and 57° F. In each experiment, four lots of chinook salmon fingerlings were subjected to constant levels of introduced ammonium hydroxide at 0.0, 0.3, 0.5, and 0.7 p.p.m., calculated as NH_4. The highest experimental level approximates the maximum concentration encountered in rearing ponds during afternoon surges.

Effects of ammonia on gills of fish held at 43° F. are shown in figures 3 to 6. Figure 3 is a photomicrograph of a typical gill section from the control group showing no proliferation of the gill epithelium. In figures 4 through 6 the correlation of proliferation with exposure to higher ammonia concentrations is obvious. These sections were selected as generally typical of the samples examined. At both temperatures some fish in all the samples from the ammonia-treated groups showed the extensive proliferation and clubbing of the filaments characteristic of the highest ammonia concentration. Sample checks at weekly intervals throughout both of the experiments indicated that the degree of proliferation was progressive for the first 4 weeks, after which no measurable increases were discernible.

There was no significant mortality during the experiments and for an additional 3 weeks afterwards. Examination of a sample of the low-temperature fish after 3 weeks in fresh water indicated that no recovery from the extensive proliferation had taken place. In the second experiment, conducted at 57° F. with larger fish, a marked recovery from the hyperplasia was noted after 3 weeks' exposure to fresh water. In the first experiment, the proliferated areas had consolidated; in the second, they had not.

In a third experiment, urea at 0.2, 0.3, and 0.4 p.p.m. was substituted for ammonia. Continuous exposure at these concentrations for a 3-week period resulted in no indication of proliferation of the gill epithelium in the fish, and the experiment was terminated. As the highest urea concentration exceeded the maximum level encountered in rearing ponds and the exposure period was beyond that when ammonia produced visible evidence of gill irritation, it was concluded that ammonia, not urea, was the toxicant.

The toxicity of ammonia solutions is determined by the amount of un-ionized ammonia present (Wuhrmann and Woker, 1949). The degree of dissociation is controlled primarily by the pH and the temperature of the solution. The toxicity tests on ammonia were conducted at water temperatures of 43° F. and 57° F. and at a pH of 7.8. In these tests the calculated exposure to un-ionized ammonia was 0.006 and 0.008 p.p.m. at the 0.3 p.p.m. ammonium hydroxide level; 0.010 and 0.012 p.p.m. at the 0.5 p.p.m. level; and 0.014 and 0.018 p.p.m. at the 0.7 p.p.m. level.

Most investigations have been concerned with the lethal effects of ammonia concentrations on the fish. Lloyd and Herbert (1960) have demonstrated that the free carbon dioxide in the water can cause a decrease in the pH value and the amount of un-ionized ammonia at the gills. Downing and Merkins (1955) and Merkins and Downing (1957) demonstrated that the lethality of un-ionized ammonia increased with a reduction in dissolved oxygen. This is consistent with the work of Phillips et al. (1949) and Brockway (1950) in which a reduction in the oxygen level of the blood was correlated with an increase in the catabolic products in the water.

Lloyd and Herbert report the lethal level of un-ionized ammonia at a pH of 8.2

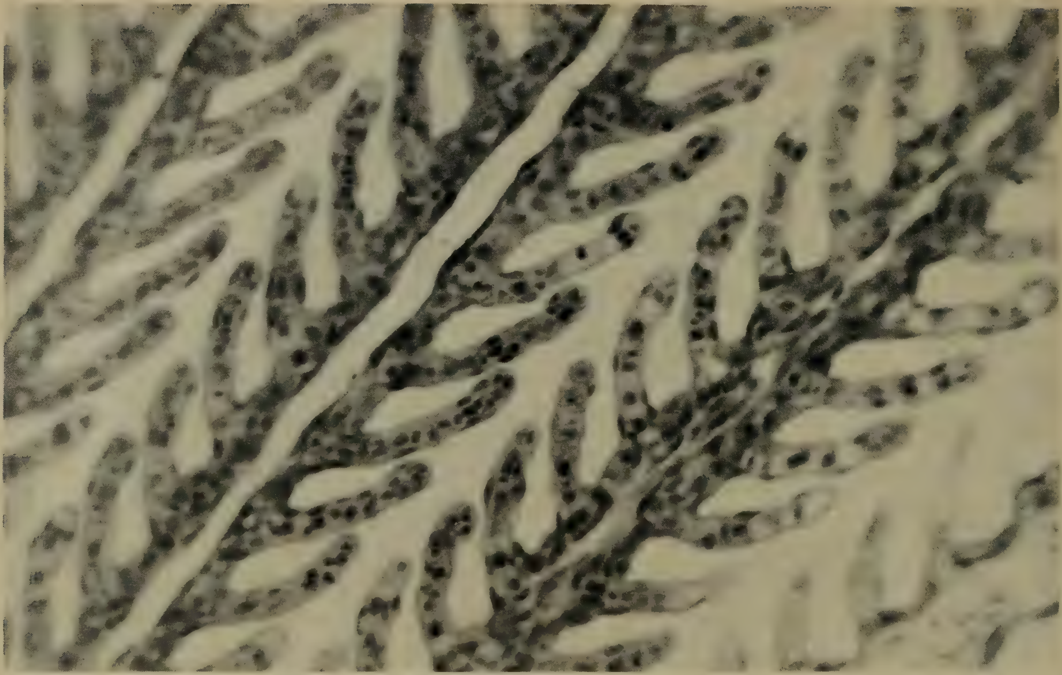

Figure 3.—Normal gill section from control group.

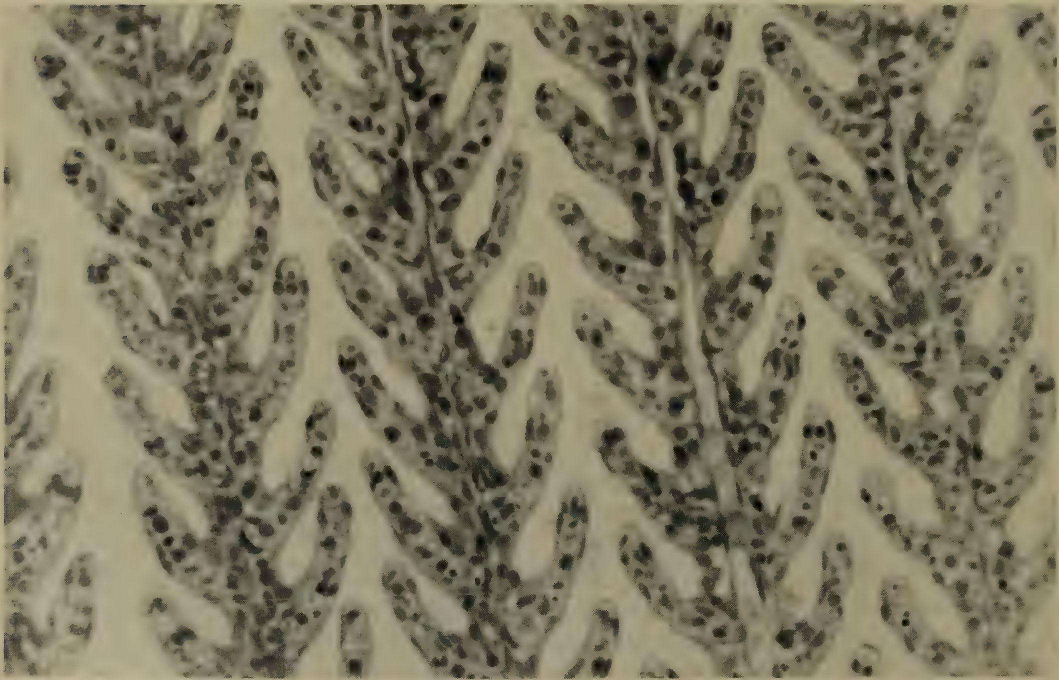

Figure 4.—Typical gill section from group exposed to 0.3 p.p.m. of ammonium hydroxide for 6 weeks showing proliferation of the gill epithelium.

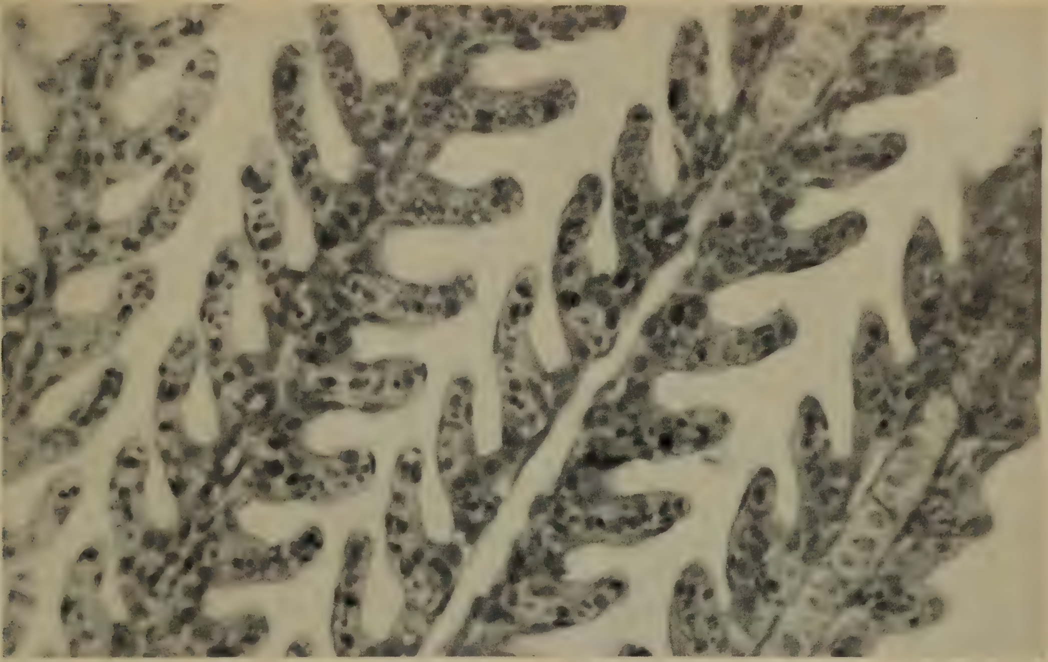

FIGURE 5.—Typical gill section from group exposed to 0.5 p.p.m. of ammonium hydroxide for 6 weeks showing extensive hyperplasia.

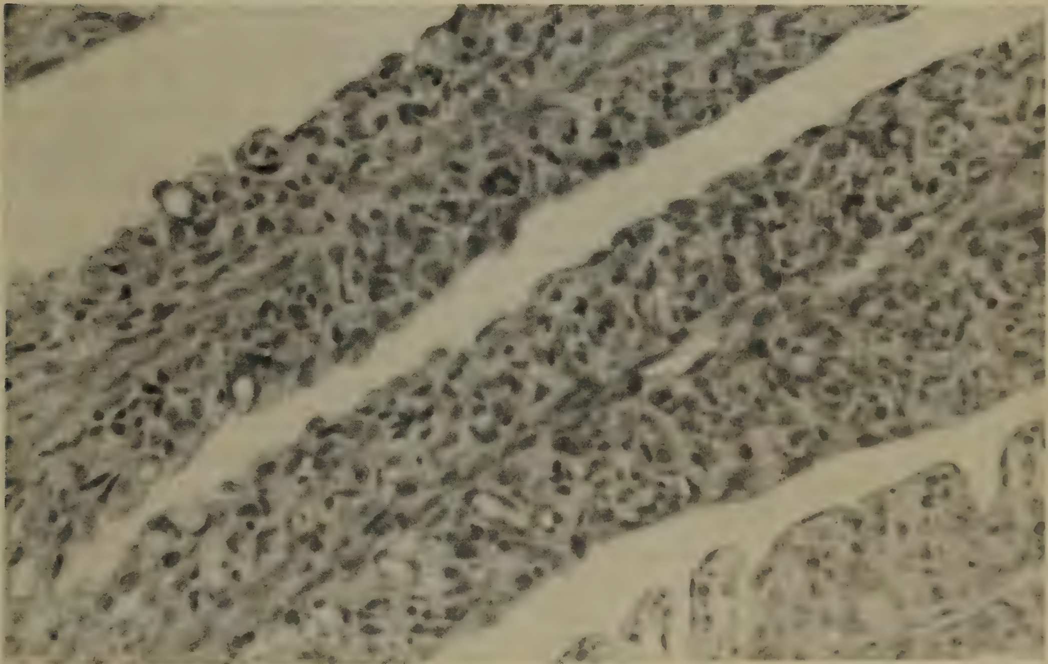

FIGURE 6.—Typical gill section from group exposed to 0.7 p.p.m. of ammonium hydroxide for 6 weeks showing fusion of lamellae. (Photomicrographs by W. T. Yasutake.)

and normal levels of oxygen and carbon dioxide to be 0.84 p.p.m. At a water temperature of 50° F., 18 p.p.m. of ammonia nitrogen expressed as NH_4 would be necessary to produce a lethal effect in rainbow trout (*Salmo gairdneri*). Such a concentration is highly unlikely in salmon and trout rearing ponds unless the water is being extensively reused without reconditioning.

The toxic levels of ammonia, other than actual lethal levels, are difficult to ascertain primarily because the effects of ammonia exposure are insidious and indirect. The literature is confusing because most authors merely give the concentration of ammonia found in the pond water, when describing toxic or lethal effects. As the toxin is the un-ionized ammonia present, the toxic concentrations cannot be calculated unless the water temperature and pH are known. Table 1 lists the percentages of un-ionized ammonia contained in ammonium hydroxide solutions at three temperatures and the pH levels normally encountered in salmonoid rearing. From table 1 it will be noted that insignificant levels of the gas are present at a pH of 7 but that the proportion of un-ionized, i.e. toxic, ammonia progressively and markedly increases with the increase of the pH of the aqueous solution, whereas the influence of temperature on the rate of dissociation is comparatively minor. The correlation between pH and un-ionized ammonia explains the vast differences encountered in the carrying capacities of different water supplies.

The effects of even low concentrations of un-ionized ammonia are real, demonstrable, and definitely detrimental. From accumulated evidence it can be demonstrated that, when un-ionized ammonia concentrations become dominant in rearing ponds, growth rate, physical stamina, and disease resistance are impaired.

TABLE 1.—*Percentage of un-ionized ammonia in ammonium hydroxide solutions at three temperatures and various levels of pH*

[Prepared by J. W. Elliott after Wuhrmann and Woker (1949)]

pH	At 10° C. (50° F.)	At 15° C. (59° F.)	At 20° C. (68° F.)
7.0	0.3	0.4	0.5
7.5	1.1	1.3	1.5
7.6	1.4	1.6	1.9
7.7	1.8	2.1	2.4
7.8	2.3	2.6	3.0
7.9	2.9	3.3	3.8
8.0	3.6	4.1	4.7
8.1	4.6	5.2	6.0
8.2	5.7	6.5	7.3
8.3	7.1	8.0	9.1
8.4	8.9	9.9	11.2
8.5	11.1	12.3	13.7
8.8	20.3	22.1	24.2
9.0	29.1	32.3	35.8
9.5	57.6	59.8	62.1

Derivation formula: $\frac{(NH_4^+)(OH^-)}{(NH_3 \cdot H_2O)} = K_b$.

K_b = dissociation constant aqueous ammonia.

K_w = ionization constant H_2O.

Temperature	K_b	K_w
10° C.	1.570×10^{-5}	14.5346
15° C.	1.652×10^{-5}	14.3463
20° C.	1.710×10^{-5}	14.1669

The reduction in growth rate due to an increase in ammonia concentration is reported from warmwater fish culture (Kawamoto et al., 1957; Kawamoto, 1958, 1961) and from salmonoid culture (Brockway, 1950). These reports are concerned with the increase in ammonia occasioned by the reuse of water and demonstrate the reduction in growth rate in ponds at the lower end of a series. In the experiment from which the excretory cycles were determined, figures 1 and 2, the growth rate was also measured. The experiment was conducted for a 5-month period with ammonia becoming dominant in the low-inflow raceway during the last 3 months. The growth rate in this raceway was 9.1 percent lower than that measured in the high-inflow raceway for the 5-month period. This significant difference occurred with but a single use of the water and without either an increase in mortality, disease outbreak, or continuous exposure to measurable levels of ammonia.

An inhibition in growth rate indicates an impaired physiology. Phillips et al. (1949) and Brockway (1950) correlate the increase in ammonia concentration with a reduction in the oxygen level in the blood. Our toxicity experiments demonstrate the extensive proliferation of the gill lamellae which can be caused by exposure to ammonia. Such proliferation and consolidation reduces the surface area of the gills and therefore the ability of the animal to absorb oxygen. These factors would be expected to result in a reduction in the physical performance of the fish under conditions of stress. Stamina trials in which groups of 100 fish were subjected to fixed water velocities for measured periods of time in a stamina tunnel (Thomas et al., 1964) demonstrated a reduced performance in fish subjected to a continuous ammonia exposure in rearing ponds as contrasted with comparable fish where the ammonia exposure was intermittent. The performance index in the ammonia-exposed fish was 16 and in the control 23. A difference of 3 performance index points was significant.

Impaired physiology implies reduced disease resistance. The susceptibility of salmon fingerlings to bacterial gill disease is closely associated with the extensive epithelial hyperplasia of the gills caused by ammonia irritation. When fish are continuously exposed to measurable amounts of ammonia, bacterial gill disease epidemics are prevalent. It is postulated that ammonia irritation is a precursor of bacterial gill disease.

The accumulated evidence indicates that continuous exposure to un-ionized ammonia is definitely detrimental and should be avoided in rearing ponds. The debilitation caused by ammonia exposure is certainly not conducive to optimum survival rates after liberation.

The principal factors affecting toxic ammonia levels in rearing pond waters are pH, temperature, and the ammonia exposure period. The fact that un-ionized ammonia is the toxin precludes the possibility of generalizing on the dangerous ammonium hydroxide levels to be encountered in rearing ponds. Within rather narrow pH ranges, however, the changes in un-ionized ammonia are relatively insignificant. Our work was conducted at a pH of 7.8±0.1, that of Phillips et al. (1950) at an initial pH of 7.8 and 7.9. This work and the summarization of the Cortland Laboratory experiments by Brockway (1950) are in essential agreement with our findings concerning the toxicity of ammonium hydroxide concentrations in rearing ponds. Our experiments indicate that the period of exposure to measurable amounts of ammonium hydroxide within a pH range of 7.8 to 8.0 is of more importance than the peak exposure concentration. Salmon fingerlings can tolerate levels of ammonium hydroxide as great as 0.7 p.p.m. for 1 hour per day with apparent impunity, but when the exposure period exceeds 12 hours per day at levels of 0.1 p.p.m. or above, the growth rate is reduced, and when continuous exposure occurs a reduction in stamina and disease resistance develops. This latter condition occurs in raceways when the fish are stocked at the rate of 8.5 pounds per gallon per minute of inflow at a water temperature of 60° F. and a pH of 7.8.

As the water temperature increases the percent of un-ionized ammonia increases and, according to Brockway (1950), the excretion rate also increases. At temperatures of 65° F., therefore, the carrying capacity of the water would be reduced to 8.0 pounds per gallon per minute of inflow or less. At pH levels above 8, the toxicity to the fish would be greatly increased and, conversely, at lower levels it would be reduced because of the variations in the amount of un-ionized ammonia present despite the production of comparable amounts of ammonium hydroxide under both conditions.

Because the length of the exposure period affects the toxicity of un-ionized ammonia, the hydraulic characteristics and explusion rates of the various types of rearing ponds assume importance. Pond types, which develop excessive detention times either due to poor hydraulic patterns or inadequate inflows, create conditions whereby the ammonia exposure is increased beyond the normal. Burrows and Chenoweth (1955) describe three pond types and the hydraulic patterns which can create such conditions and have correlated the hydraulic characteristics with the disease inhibition qualities. Brockway (1950) emphasizes the effects of laminar flow in raceways with inadequate inflow and was able to demonstrate increased ammonia concentrations in the deeper stratified areas.

Recognition of the period when dangerous toxic levels of ammonia are approaching is essential if they are to be avoided. Examination of the excretory patterns indicates that ammonia as it approaches dominance is present in the p.m. hours and urea in the a.m. hours. As the period of ammonia excretion increases in the 24-hour cycle, the low ebb occurs in the early morning. The occurrence of measurable amounts of ammonia in the pond effluent during the period of minimum physical and metabolic activity is an indication that the fish are being subjected to a continuous ammonia exposure and an unfavorable environment.

These investigations have demonstrated that continuous exposure to un-ionized ammonia can produce a general debility. Such debility may be an important factor influencing survival of salmon and trout both before and after liberation.

CONCLUSIONS

Experiments conducted to determine the excretory products of significance in salmonid rearing ponds and the effect of these products on the fish lead to the following conclusions:

1. Chinook salmon fingerlings excrete principally ammonia and urea.

2. The patterns of excretion and composition of the excretory products over a 24-hour cycle vary with the density of the fish. At stocking rates of less than 5 pounds per gallon per minute of inflow, urea is the principal excretory product. Above this level ammonia becomes dominant.

3. Concentrations of un-ionized ammonia as low as 0.006 p.p.m. in continuous exposure for 6 weeks can produce extensive hyperplasia of the gill epithelium.

4. No deleterious effects were discernible when chinook fingerlings were subjected to continuous exposure to urea for 3 weeks at levels normally encountered in rearing ponds.

5. Controlled experiments have demonstrated that prolonged but intermittent exposure to un-ionized ammonia results in reduced growth rate and physical stamina. It is postulated that continuous ammonia exposure is the precursor of bacterial gill disease.

6. The pH and water temperature determine the amount of un-ionized ammonia present; the pond type and the

loading rate in relation to water inflow determine the duration of the exposure period.

7. Where ammonia is a problem, dangerous levels are indicated by the presence of measurable amounts in the effluent during the low ebb of the diurnal excretory cycle.

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A DEVICE FOR STAMINA MEASUREMENT OF FINGERLING SALMONIDS

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RESEARCH REPORT 67

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A DEVICE FOR STAMINA MEASUREMENT OF FINGERLING SALMONIDS

Allan E. Thomas, Roger E. Burrows,
and Harry H. Chenoweth

RESEARCH REPORT 67

UNITED STATES DEPARTMENT OF THE INTERIOR
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ABSTRACT

A stamina tunnel was developed to measure differences in physical performance of salmonid fingerlings. By subjecting fish samples to controlled patterns of water velocity, it has proved possible to demonstrate differences in fish stamina imparted by disease, nutrition, and environment.

A DEVICE FOR STAMINA MEASUREMENT OF FINGERLING SALMONIDS

By Allan E. Thomas and Roger E. Burrows, ***Fishery Research Biologists,***
and Harry H. Chenoweth, ***Hydraulic Engineer***

Salmon-Cultural Laboratory, Bureau of Sport Fisheries and Wildlife
Longview, Washington

One of the principal problems of artificial propagation is evaluation of the quality of the fish produced. The physical stamina of the fingerling is assumed to be one measure of the ability of the fish to survive after release. A device for the measurement of physical stamina must meet certain requirements to be of practical value in any large-scale evaluation of hatchery production:

1. The device should be capable of rapidly testing representative samples of hatchery populations.
2. It should be able to reproduce exactly a measured stress.
3. It should be capable of imposing increased stress to conform with increased ability.
4. It should have sufficient sensitivity to measure the stamina differences created by the varying conditions encountered in artificial propagation.

Several methods have been employed to measure the swimming ability of fish. The most popular type of apparatus seems to be a rotating annular trough or "fish wheel." Adaptations of this idea have been used by Bainbridge and Brown (1958), Paulik and DeLacy (1957), Gray (1957), Brett et al. (1958), and others. One fish at a time is tested. The velocity of the water in the trough is essentially that of the trough, although is some designs slippage may occur. The introduction of cages, electrodes, and similar devices for stimulation of the fish increases the slippage between water and trough. If larger samples of fish are to be tested simultaneously, the trough must be wide. The radius must be large if the difference between the inner and outer velocities is to be negligible. The annular trough may also produce a laminar flow of water which may have a different effect on the swimming ability of the fish than would more turbulent water.

Open, straight troughs, such as used by Reimers (1956) and Vincent (1960), and the flume used by Kerr (1953) have the disadvantage of a substantial difference in water velocities within the trough. If the bottom is rectangular, the velocity in the lower corners will be low. Without closely controlled water velocities, fish tested will not all be subjected to the same swimming requirements.

The method of frightening fish for a fixed time interval to produce exercise, as used by Miller et al. (1959) and Hochachka (1961) may result in nonuniform treatment of fish samples. "Closed-circuit" water tunnels such as are described by Mar (1959), Katz et al. (1959), and Davis et al. (1963) have the advantages of closely controlled water velocities and more nearly uniform treat-

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The authors wish to receive equal credit for this publication.

ment of fish samples. Under most conditions only a few fish can be tested at one time.

None of the devices described in the literature meets the requirements for large scale evaluation of stamina in hatchery fish. Our stamina tunnel was designed to meet these requirements. It has been tested over a period of 4 years with thousands of fish samples from different sources and has proved adequate for stamina measurement. By recognition and control of extraneous variables it has been possible to measure fish performance differences imparted by disease, nutrition, and environment.

EXPERIMENTAL APPARATUS

The stamina tunnel was designed specifically for stamina measurement. In these measurements the search is for differences imparted by artificial propagation and not precise determinations of swimming speeds. The tunnel is comparatively large in order to test large samples. Control of water velocities is exact and reproducible with but minor error. The velocities available are in excess of the requirements for testing small fingerlings and thereby provide reserve velocities for the imposition of additional stress. Design drawings of the tunnel are shown in figure 1. In this tunnel the water is

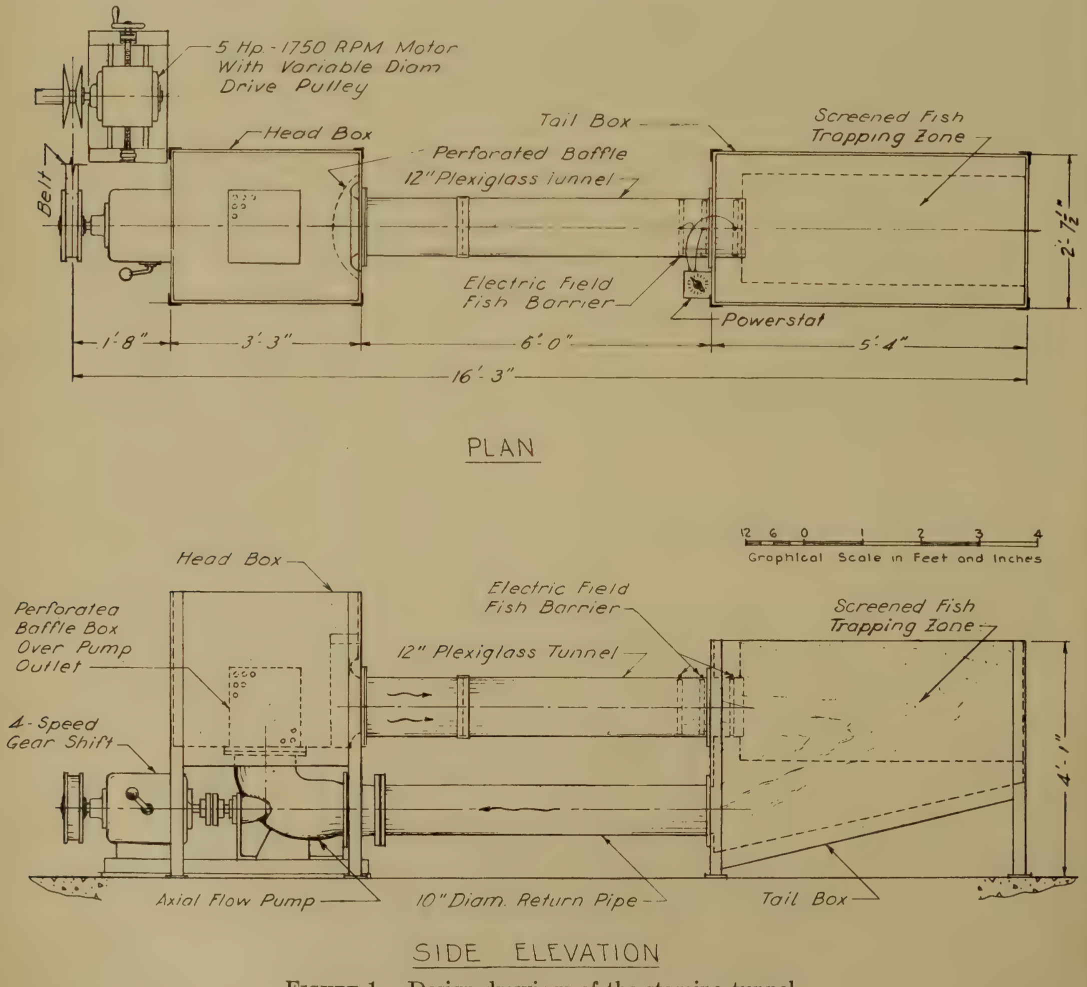

FIGURE 1.—Design drawings of the stamina tunnel.

recirculated by means of a 1,500 gallon per minute axial flow pump. A four-speed transmission and variable-speed drive pulley are combined to allow a wide range of water velocities to be produced. Speeds can be varied within each of the four ranges while the pump is running.

The discharge from the pump passes through perforated baffles upon entering the head box. These baffles are designed to provide adequate diffusion at both high and low velocities. The holes of the baffles are small so that the resulting turbulence will be fine and therefore will dissipate quickly. The size of the holes in these baffles will depend on the water velocities desired for the tunnel.

The head box is adequate but is near the minimum size necessary to meet the capacity of the pump. Two perforated plates are located at the entrance to the tube, the first to produce a more uniform flow and the second to confine the fish.

The tunnel consists of a 12-inch outside diameter plexiglass tube, 6 feet in length. The flow through the tunnel is controlled by a differential in water level between the head and tail boxes. The fixed cross-section of the 12-inch tube makes the velocities through the tube a direct function of the mass flow rate of the pump. For a fixed water path this flow is a function of pump speed. The rotational rate of the pump can easily be varied and measured.

A uniform velocity at the entrance of the tunnel is secured by the well-rounded bell mouth. A considerable fluid acceleration takes place at the tunnel entrance. It is this acceleration which creates the uniform velocity. The round cross-section helps to maintain this uniform velocity although any boundary will retard the motion of the adjacent flow. The final velocity profile is obtained 50 pipe diameters downstream from a well-rounded intake in a long pipe. Since this water tunnel has a length of only 6 pipe diameters (6 feet), the velocity profile in the tunnel is changed only slightly throughout its length. Typical velocity profiles obtained at the tunnel outlet are shown in figure 2. The velocity in the upstream test section will be even more uniform because the boundary layer has had less opportunity to slow down the flow near the wall.

An electrical field fish barrier is located near the outlet of the tunnel. Three stationary aluminum bands are positioned inside the tunnel. Electrical fields are created between the positive middle band and negative bands on either side, and the strength of the fields is controlled by a variable transformer.

Fish leaving the tunnel during a stamina test are held in a screened trapping zone or collection chamber of the tail box. The water diffuses through the screens to the tail box sump, from which it enters the return pipe to the pump.

Energy is added to the fluid on each pass through the pump. This energy eventually is converted to heat. The computed rise in the water temperature with a tunnel velocity of 2 feet per second was under 0.5° F. per hour of continuous operation. This insignificant rise is masked by heat transference from the air and is corrected between stamina tests by the addition of more water at 53° F. temperature.

The design of the tunnel has proved very satisfactory for stamina testing of fingerling salmon. No alterations in design have been found desirable.

TESTING PROCEDURE

In the normal procedure of stamina testing, the fish are segregated into groups, temperature acclimated, tested in the tunnel, and weighed after testing. Samples from the lot to be tested are randomly distributed into groups of 100 fish each. These samples are held sepa-

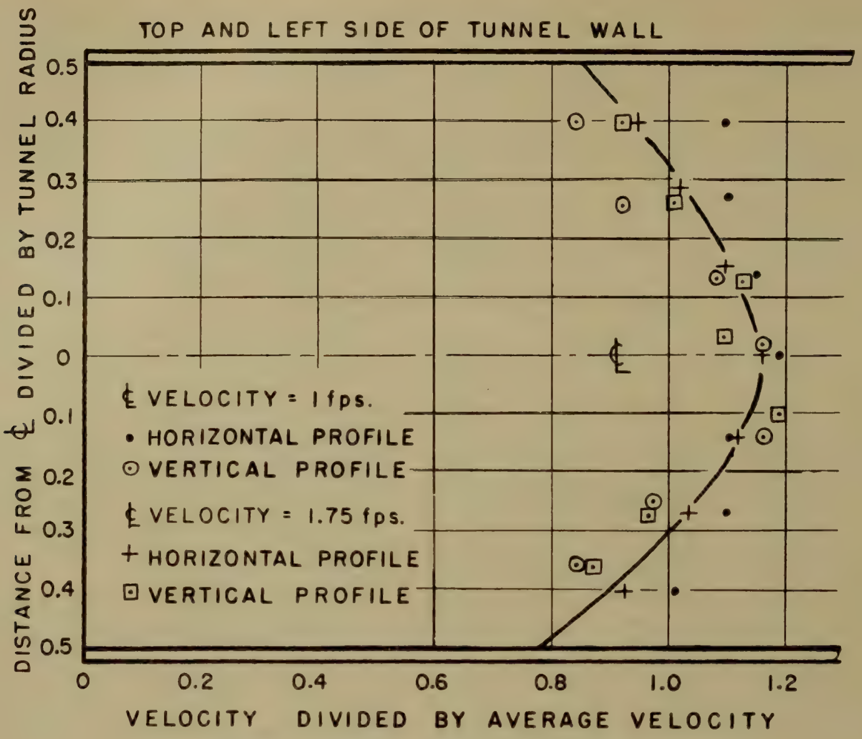

Figure 2.—Velocity profiles at tunnel outlet.

rate and acclimated to 53° F. water for a period of 16 to 20 hours. The fish are not fed during this time. At the end of the temperature acclimation period, the group of fish is introduced into the clear-plastic tunnel by means of a removable loading chute. After a brief orientation period, a procedure of increasing the water velocity by increments is begun.

The size of the tunnel is such that a standard sample of even large fingerlings is not crowded. The distribution of fish in the tunnel is shown in figure 3. Under test conditions, the tunnel is covered during operation to reduce the possibility of outside disturbance and to allow the fish to orientate within the tunnel.

The electrical field at the outlet induces the fish to remain in the tunnel until partially exhausted. When they can no longer maintain their position against the water current, the individual fish are swept through the electrical field into the tail box where they are held until the end of the test. The voltage used in the electrical field varies from 7 to 12 volts with the setting depending upon the size of fish tested. The lower voltage is used for the larger fingerlings.

The water velocities and exposure times under two operating procedures of stamina testing are shown in figure 4. Fish ranging from approximately 1 to 4.5 grams per fish are normally tested in second gear at water velocities ranging from 0.45 foot per second to 1.70 feet per second. Larger fingerlings are usually tested in third gear with water velocities from 0.75 foot per second to 2.70 feet per second. Smaller fish are given an advantage of less initial velocity, which is not necessary for larger fish. The testing procedure gives two identical velocity

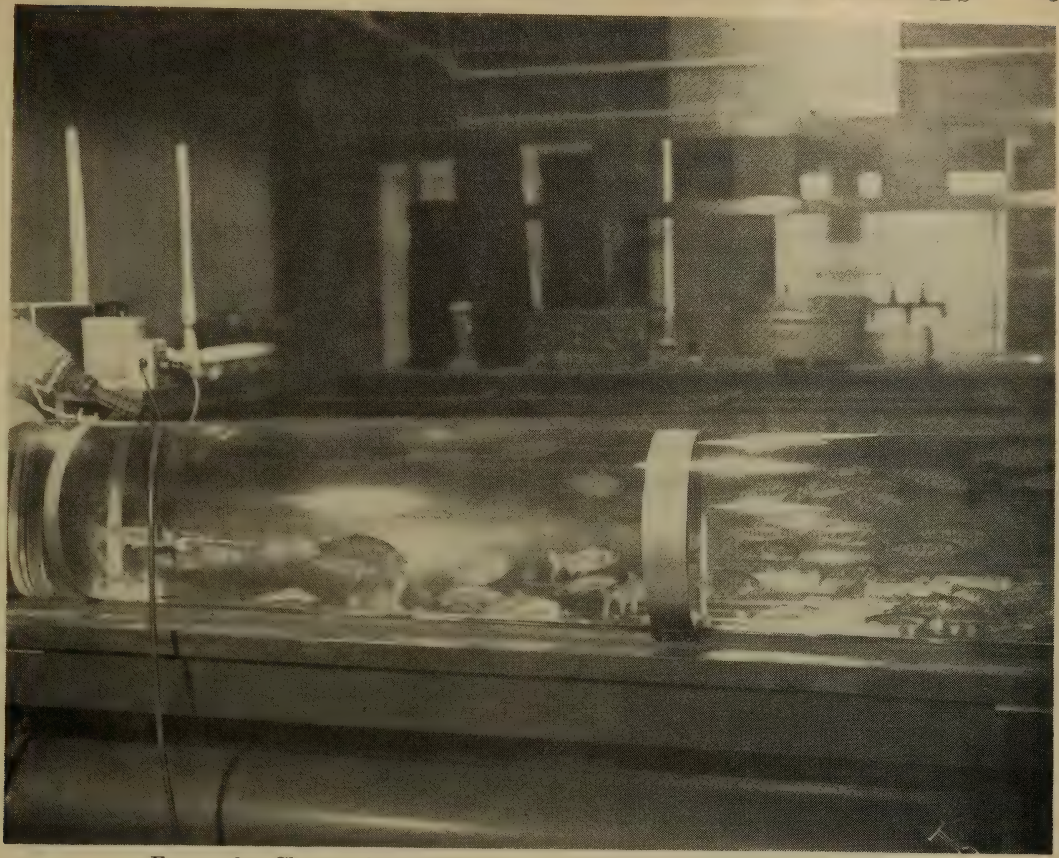

FIGURE 3.—Closeup showing the normal distribution of fish in the plexiglass tunnel at a low water velocity.

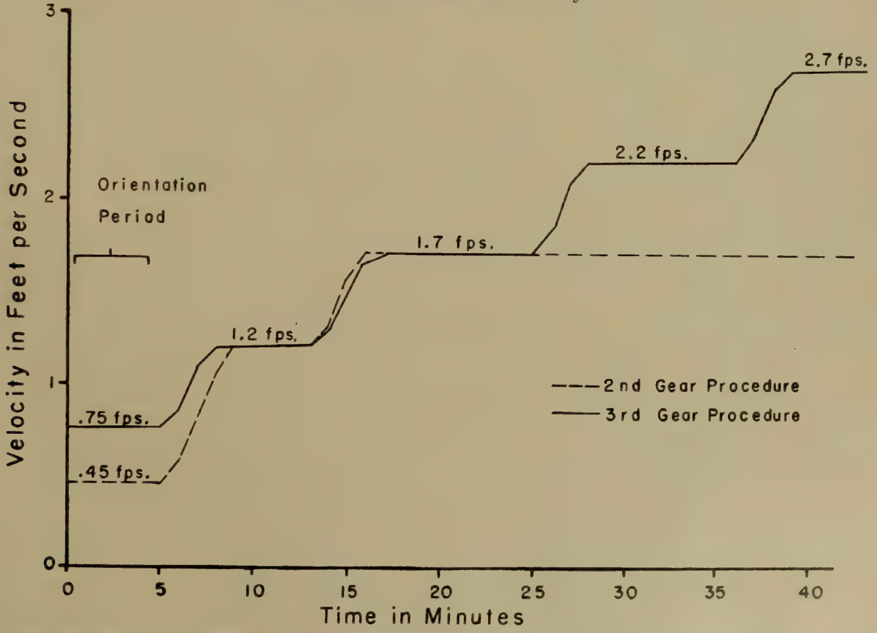

FIGURE 4.—Water velocities and exposure times for stamina testing using two gear speeds.

plateaus of 1.2 feet per second and 1.7 feet per second for both gears, and two additional plateaus of 2.2 feet per second and 2.7 feet per second for the third gear.

The fish are counted as they leave the tunnel, and the cumulative totals are recorded at 1-minute intervals. The stamina test is completed when at least 75 percent of the fish have left the tunnel. At the conclusion of each test the total weight of each sample is determined.

PERFORMANCE INDEX

A performance index has been developed for the stamina measurement of comparable fish samples. This index is the summation of the times when 25 percent and 75 percent of the fish leave the tunnel. The index measures the swimming ability of only the middle 50 percent of the fish tested and eliminates the strongest and weakest fish. The elimination of these fish keeps to a minimum any possible effect due to the abnormal performance of a few fish.

The performance index has proved valuable in a great number of stamina tests as a rapid and reliable method of measurement. Fish size, however, affects swimming ability and therefore the performance index. A normal performance curve can be established for a population of fish by conducting a series of tests during different periods of their growth. The existence of differences between fish populations can then be determined by comparing the performance indexes with the established values for fish of the same size. In past testing, a difference in the performance index of about three points between fish of the same size has usually been a significant difference.

VARIABLES IN TESTING

There are a number of controllable variables which must be considered if testing is to be standardized. These controllable variables are size of fish, water temperature, oxygen content, sampling technique, size of sample, starvation period, frequency of retesting, and species of fish.

Size of fish

Under normal conditions the performance of fish improves with an increase in size. Kerr (1953) in testing the effects of water velocity on juvenile striped bass (*Roccus saxatilis*) and chinook salmon (*Oncorhynchus tshawytscha*) found an increase in swimming ability with larger fish and little difference between the ability of comparable yearling salmon and bass. Bainbridge (1960) found an increase in the swimming speed of goldfish (*Carassius auratus*), dace (*Leuciscus leuciscus*), and trout (*Salmo irideus*) with an increase in fish length.

That the swimming ability of fish increases with an increase in size is demonstrated in figure 5. The data for this graph were obtained from a series of stamina tests on fall chinook salmon fingerlings conducted at biweekly intervals during a rearing season. Five hundred fish were tested at each interval, and a total of 9,000 fish were used to determine the normal performance curve.

Water temperature

The swimming ability of fish has been found to be affected by the temperature of the water. Brett et al. (1958) found that young coho salmon (*O. kisutch*) increased their cruising speed with increases in temperature up to their ultimate lethal temperature, whereas the cruising speed of young sockeye salmon (*O. nerka*) reduced when the lethal temperature was approached. Fry (1958) reports that the cruising speed of young goldfish reaches a peak and then decreases when higher temperatures cause a stress that increases the standard metabolic rate.

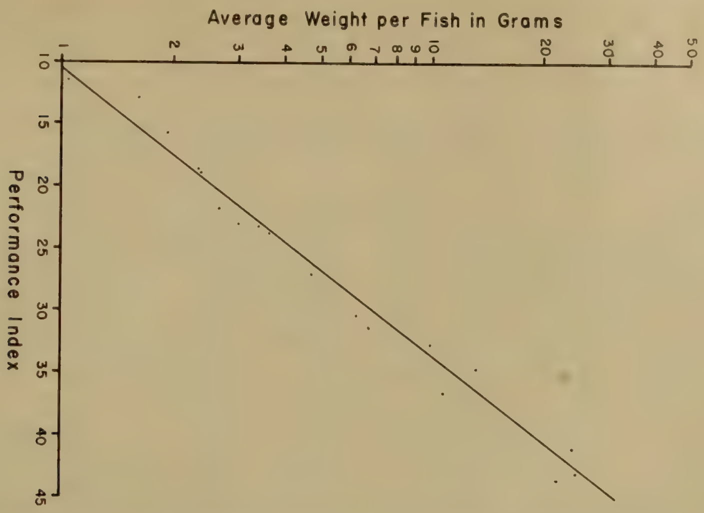

FIGURE 5.—Increase in swimming ability with increase in weight in fingerling fall chinook (*O. tshawytscha*).

The effects of the water temperature on metabolic rate of the fish are complex. Brett (1956) states that fish performance is best in the region of the "preferred temperature" and that temperatures above and below that range produce adverse results. Brett (1959) reports that sockeye salmon increase their swimming speed with increased water temperature until about 60° F. is reached. Above this temperature, he found, swimming speed is reduced until the speeds at 50° F. and 66° F. are approximately equal. The increased metabolic demand at the elevated level, however, would cause the energy reserves to be exhausted 1.5 to 2 times as fast at 66° F. as at 50° F.

To eliminate the temperature effect, stamina tests are normally conducted with acclimated fish at a constant water temperature of 53° F. This temperature gives a common base from which to compare populations of fish with different dietary and environmental regimens as well as with wild and hatchery fish from other geographical areas.

Temperature experiments in the stamina tunnel indicate that the temperature of water at time of testing has a definite effect on the swimming ability of the fish. During 1960, tests on summer chinook fingerlings were conducted at water temperatures ranging from 47.5° F. to 60° F. Fall chinook fingerlings have been tested at temperatures from 38° F. to 54.5° F. Within these limits, better performance in the stamina tunnel has resulted from increased water temperature.

In addition to the temperature effect on fish physiology, there is a change in physical resistance due to changes in water viscosity. The effect of temperature on the viscosity of the water is shown in figure 6. Both the physical and physiological conditions created by cold water temperatures result in reduced performance, and these factors must be recognized in performance evaluations.

Oxygen content

In studies by Davis et al. (1963) and Katz et al. (1959), reductions in oxygen concentration resulted in corresponding reductions of the maximum sustained swimming speeds of coho and chinook juvenile salmon. In the stamina tests, fish performed in water at 90 percent of ox-

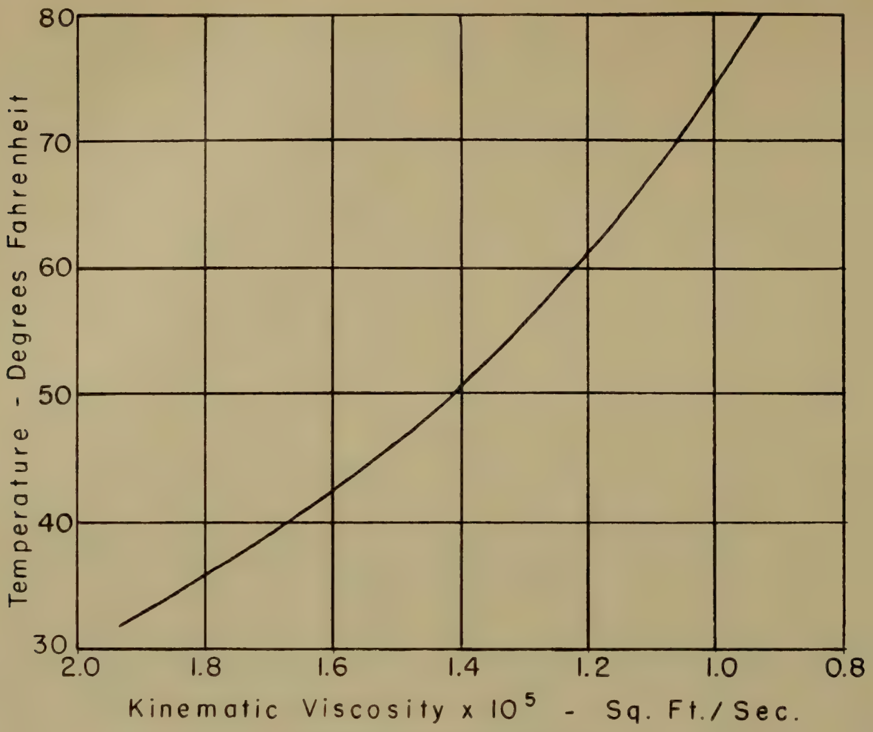

FIGURE 6.—Viscosity of water.

ygen saturation. Replenishment of water between tests assured standard conditions without the depressing effect of an oxygen deficiency.

Sampling technique

The biological variation among fish from the same diet or rearing environment is such that random samples of the fish population are necessary to give reliable data. The entire population must be handled and systematically sampled with a vertical sampler. The variables to be encountered in methods of sampling are described by Hewitt and Burrows (1948). Dip net samples may be necessary to test the progress of long-term experiments, but the results will be biased. Under such circumstances the samples should be taken either during feeding when the fish are congregated in a small area or when the fish are seined into a small section of a pond. These procedures do not permit the selection of true samples but are the best possible compromise.

Number of fish

Samples varying from 13 to 264 fish have been tested in the tunnel, with a trend towards better performance in the smaller samples. Samples of the same numbers from one population of fish perform similarly. Fish are tested in 100-fish groups to reduce the bias from biological variation which might result from fewer fish and to facilitate the evaluation of results. Standardization of the sample size rather than the actual number of fish employed appears to be the important factor in comparing populations. From two to five stamina tests of 100 fish each are normally conducted for each population to allow statistical analysis of the results.

During early testing of fish with the stamina tunnel, the question arose whether the first fish out of the tunnel were actually the weakest fish, and the last ones the strongest, or were they simply crowding each other out. In several groups of fish, the first and last 25 percent of the fish coming out of the tunnel were separated during the test and the quarters were individually retested. The quarters closely repeated their original levels, disproving any crowding effect.

Effect of feeding

As mentioned earlier, samples of fish to be tested are usually not fed for at least 16 to 20 hours before being tested. Fish which are tested within a few hours after being fed perform poorly in the tunnel.

Tests on the effects of starvation on performances, using fall chinook fingerlings averaging 1 gram per fish, resulted in no decrease in swimming ability until after 5 days without food. Fall chinook fingerlings averaging about 3 grams per fish had no performance decrease for at least 4 days of starvation. Tests on summer chinook fingerlings averaging about 15 grams per fish showed no difference in performance for at least 40 hours without food.

It would appear that, once the initial digestive period is past, the energy requirement for normal swimming can be met for a period of several days. In any study combining stamina testing with other evaluations of a population which might be influenced by starvation, a fixed time interval between testing and feeding should be established.

Effect of retesting

In some instances, such as a series of tests conducted on a small population of fish, it might be necessary to run stamina tests on fish of which all or part had previously been tested. Preliminary experiments on retesting, using summer chinook fingerlings, showed a substantial decrease in performance when immediately retested. Retesting of the fish after a rest of 24 hours produced a significant reduction in swimming ability below the initial level. In tests with fall chinook fingerlings, paired groups of 100 fish each were retested after resting periods of 1, 2, and 3 days. After 3 days of rest, the performance had returned to the previous level.

Species of fish

Most stamina tests have been conducted with hatchery-reared fall chinook salmon. Summer chinook and rainbow trout (*S. gairdneri*) fingerlings as well as spring chinook and steelhead yearlings have also been tested from hatchery stock. Tests have been conducted on spring chinook, steelhead, and coho salmon yearlings from rearing ponds. Wild fish tested in the tunnel include both fingerling and yearling fall chinook and coho salmon as well as spring and summer chinook and steelhead fingerlings.

Stamina tunnel tests indicate that fall chinook fingerlings, generally, have a higher performance level than spring and summer chinook fingerlings of the same size. This may indicate an inherent difference between the races. Collins (1958) measured the performance of adult chinook salmon in fishways and found that fall chinook were faster and more consistent swimmers than springs.

In a few tests of wild spring chinook from the Entiat River in Washington, fewer fish came out of the tunnel during the early part of the stamina test than in tests of hatchery samples of the same weight; hatchery fish of the same age but slightly larger showed the same or better performance levels. Samples of wild fall chinook gave considerably lower results than hatchery fish; these wild fish had come from an eddy area of the Cowlitz River in Washington and were probably not typical specimens. Coho salmon fingerlings were found to have slightly less swimming ability than fall chinook. Steelhead fingerlings were able to outperform comparable fall chinook. Wild coho salmon yearlings gave a poor performance owing to malnutrition, but had a 50-percent increase after being fed for 3 weeks in the hatchery, and doubled their original performance after 6 weeks of feeding.

Studies by Paulik and DeLacy (1957) on adult steelhead and coho salmon from the same stream and under identical conditions demonstrated that steelhead could outperform coho at high water velocities. Performance of sockeye salmon (Paulik and DeLacy, 1958) reduced as the distance from the ocean increased. These studies show that there are hereditary differences in the swimming ability between species of fish which persists into the adult form.

A question may arise as to the validity of comparing the performance of fish at different stages of their behavioral development, that is, comparing fish which are migrating downstream with those at earlier stages. Young steelhead, spring chinook, and coho salmon, when trapped by downstream migrant traps and later tested in the stamina tunnel, lost their tendency to migrate and swam against the current in the typical manner. This regression to an earlier stage is explained by Hoar (1958). His work on fish behavior has shown that a change in the natural sequence of stimuli, such as a barrier to migration, may cause "fall-back phenomena" to an earlier stage.

DEVELOPMENT OF A METHOD OF PERFORMANCE RATING

A method of evaluation was sought which would compensate for some of the uncontrollable variables encountered in stamina testing and thereby enable the assignment of a relative standing for performance comparison. The work done or energy expended by the fish in overcoming fluid resistance in the tunnel was selected as the logical method of performance measurement. In its simplest form, the work done is expressed in terms of force and distance. The drag forces encountered, the varying velocities imposed during the stamina trials, and the length of time the fish are capable of maintaining themselves in the tunnel, all are factors affecting the energy expended. These factors may be measured in the tunnel, or the effects may be calculated from accepted hydraulic formulas.

The drag force on an immersed body is usually expressed as $D = C_d APV^2$ where C_d is a coefficient that depends upon the shape of the body and upon Reynolds number, A is the frontal area of the body, P is the mass density of the fluid, and V is the relative velocity between the body and the fluid. Reynolds number is a similarity criterion indicating the relative importance of inertial and viscous forces on the flow pattern. For fish, Reynolds number would logically be $N_r = VL/v$ where L is the length of the fish and v is the kinematic viscosity of the water at the existing temperature (fig. 6).

For fish of the same general shape, equal Reynolds numbers would indicate similar flow patterns about the fish. Kinematic similarity is said to exist about two immersed bodies if the ratio of velocities at all corresponding points is constant. If the local water velocity past a fish is 1½ times the speed of a small fish, it is also 1½ times the speed of a large fish if the Reynolds numbers are equal and geometric similitude exists.

The drag on an immersed body arises from two sources, shearing stress and normal stress. The first of these is called frictional or surface resistance, and the second is called form drag. Frictional drag on fish can be represented in a manner similar to that of a flat plate pushed through a fluid with the plate parallel to the direction of motion. Gray (1957) suggests that this is a reasonable assumption. Form drag can be reduced by streamlining or by devices that control boundary layer growth on the immersed body. A fish is highly streamlined, and his drag is essentially due to frictional or surface effects.

For values of Reynolds number below about 400,000, the coefficient of drag may be expressed as $C_d = \frac{0.7}{\sqrt{N_r}}$. Fish within a species have geometrically similar bodies, and the surface area varies with the square of the length of the body. Substituting these expressions into the drag equation yields $D = C_1L^{3/2}PV^{3/2}v^{1/2}$ where C_1 is a proportionality constant whose numerical value could be determined. The effects of water temperature and fish size on the resistance to motion and the energy expenditure required in overcoming this resistance can now be deduced.

An example of resistance would be fish moving through water at 35° F. and then through water at 82° F. The fluid is approximately twice as viscous at 35° F. as at 82° F. At the lower temperature, the fish would find it much more difficult to attain high speed. Reference to the last equation shows that for the same speed the water resistance to motion would be 41 percent greater at 35° F. than at 82° F.

The next consideration is the effect of size on resistance and power. A 10-centimeter and a 20-centimeter fish can be used for illustrative purposes. If swimming in the same water at the same speed, the drag equation indicates that the resistance to motion of the larger fish will be 2.83 times that of the smaller fish, the power required will also be 2.83 times that of the smaller fish. This additional resistance should not tax the larger fish. His volume and weight are about eight times that of the shorter fish and he should have the musculature and energy necessary to reach a correspondingly greater speed.

If output power is proportional to weight, the 20-centimeter fish should swim at about 1.5 times the speed of the 10-centimeter fish. Substitution of these lengths and velocities into the drag equation will show that resistance to motion under this latter condition is 5.3 times as great for the larger fish as for the smaller fish. The work required is the product of resistance and velocity and is eight times as great for the larger fish as for the smaller one.

The work performed by a swimming fish moving with variable speed through a series of increments with constant velocity during each increment is expressed as follows: Work $= \Sigma\,(D_1S_1 + D_2S_2 + D_3S_3 \ldots.) = \Sigma\, D_iS_i$. Here D_i is the drag force during the increment and S_i is the distance swum during the increment. Since the distance traveled by the fish during the increment of contant velocity is equal to the velocity multiplied by the length of the time interval T_i, Work $= \Sigma D_iV_iT_i$.

The equation for drag force was earlier written as D $= C_2L^{3/2}PV^{3/2}v^{1/2}$. For water near 54°F. the viscosity can be approximated by the formula $v = 54/\text{F.}^\circ\ (1.3 \times 10^{-5})$. Making this substitution into the drag equation gives $D = C_2L^{3/2}PV^{3/2}\,(54/\text{F.}^\circ)^{1/2}$. The work equation can now be written: Work $= C_2L^{3/2}P(54/\text{F.}^\circ)^{1/2}\ \Sigma\, V_i^{5/2}T_i$.

All the variables on the right side of the above equation can be evaluated by measurements. The method of operating the stamina tunnel allows for the determination of $\Sigma\, V_i^{5/2}T_i$. The effect of fish size and water viscosity can be calculated.

The muscle power of the fish must be based on a set of assumptions. A common and reasonable assumption is that the swimming ability of a fish should be proportional to his muscle weight. Gray (1957) goes so far as to estimate the constant of proportionality as 0.002 horsepower per pound of body weight. Two other assumptions to be made are: (1) muscle weight is some fixed fraction of the body weight, and (2) fish weight varies as the cube of the fish's length. The last assumption has been investigated by Le Cren (1951), who found the exponent to be between 2.5 and 4.0 for perch, and by Bainbridge (1960), who found the exponent to be 3.2 for goldfish, 3.0 for trout, and 2.8 for dace. Based on the three stated assumptions, the ratio of useful work done to the muscle power available for this purpose may be expressed in the equation: Performance rating $= C_3\sqrt{\frac{54}{\mathrm{F.}}\times\frac{1}{\mathrm{Wt.}}}\ \Sigma V_i^{5/2}T_i$.

Since only relative performance is meaningful, the constant C_3 can be arbitrary as long as it is applied to all samples. A constant of 174 has been used with fish tested in the stamina tunnel as it places the expected performance of chinook salmon fingerlings reared in raceway ponds at 100.

The portions of the sample selected for evaluation in the performance rating differ from those used in the performance index. In energy measurements the energy expenditure varies as the square of the velocity. In the tunnel tests fish are subjected to periodic increases in velocity. As the variation in swimming ability can vary considerably within a sample, it is entirely possible for the excellent performance of a few fish to obscure the poor performance of the majority of the lot. To correct for this possible distortion, the ratings are based on the cumulative energy expenditure of 50 percent and 75 percent of the sample.

A high value for the performance rating does not necessarily mean that a fish has expended a large amount of energy. It does indicate that the fish performed well compared with what would have been expected from him. The rating should not be applied to fish over 50 cm. long because the flow pattern around such fish is not likely to be similar to that used in the derivation of the formula.

Table 1 lists the calculated values for the energy expenditure measurement, $\Sigma V_i^{5/2}T_i$, calculated for the regimen used in the normal testing procedure of the stamina tunnel. If identical water velocities and exposure times are used in other tunnels the table values will be applicable.

Table 1.—Calculated values for $\Sigma V_i^{5/2}T_i$ under normal testing procedure.

Time	$\Sigma V_i^{5/2}T_i$	
	Second-gear procedure	Third-gear procedure
1 minute	0.136	0.488
2 minutes	0.272	0.976
3 minutes	0.408	1.464
4 minutes	0.544	1.952
5 minutes	0.680	2.440
6 minutes	0.930	3.130
7 minutes	1.550	4.340
8 minutes	2.750	5.920
9 minutes	4.330	7.500
10 minutes	5.910	9.080
11 minutes	7.490	10.660
12 minutes	9.070	12.240
13 minutes	10.640	13.810
14 minutes	12.490	15.670
15 minutes	15.020	18.200
16 minutes	18.360	21.540
17 minutes	22.130	25.310
18 minutes	25.900	29.080
19 minutes	29.670	32.850
20 minutes	33.440	36.620
21 minutes	37.210	40.390
22 minutes	40.980	44.160
23 minutes	44.750	47.930
24 minutes	48.520	51.670
25 minutes	52.290	55.470
26 minutes	56.060	60.590
27 minutes	--------	66.840
28 minutes	--------	73.990
29 minutes	--------	81.140
30 minutes	--------	88.290
31 minutes	--------	95.440
32 minutes	--------	102.590
33 minutes	--------	109.740
34 minutes	--------	116.890
35 minutes	--------	124.040
36 minutes	--------	131.190
37 minutes	--------	139.390
38 minutes	--------	149.990
39 minutes	--------	161.990
40 minutes	--------	173.990
41 minutes	--------	185.990
42 minutes	--------	197.990

FACTORS MEASURED BY THE TUNNEL

It has been demonstrated that the stamina tunnel can measure differences in the performance of fish affected by disease, imbalanced diets, or different environments. In the evaluation of fish affected by disease, infections of the gills resulting in extensive proliferation of the epithelial layer or fusion of the lamellae and filaments produce the most obvious reductions in performance. Other infections causing general debilities are readily measurable by inferior performance. Salmon fingerlings with hematocrits below 30 percent perform poorly in the tunnel. Excessively fat fish have a lowered stamina. Rearing ponds which develop sufficient water velocity to exercise the fish produce fingerlings with superior stamina. Fish from overcrowded ponds have reduced ability to perform. All of these differences may be imposed by variations in methods of artificial propagation. If such physiological differences affect the potential of the fingerlings for survival then the measurement of physical stamina by means of the tunnel may prove to be a valid measure of the survival potential of the fingerling at time of release. Experiments are in progress to determine whether stamina as measured by the tunnel is a factor influencing adult survival.

DISCUSSION OF EVALUATION METHODS

The methods of evaluation developed for the stamina tunnel are the performance index and the performance rating. There are advantages and disadvantages in the use of either method.

The performance index is derived from an easily calculated empirical formula and represents the cumulative time that parts of a sample remain in the tunnel. It does not account for the effect of either fish size or water temperature. In the evaluation of fish of different sizes, a standard curve should be established for a population of fish during a rearing season, and the performance indexes of other samples should be compared with that curve. The performance level is also dependent upon the temperature of the water, and the interpretation of results from tests in varying water temperatures would be difficult.

The performance rating is based on the energy expended by the fish and compensates for both the weight of the fish and the viscosity of the water. It expresses the relation of a sample to the normal or expected performance of fish of that size regardless of the water temperature at which the fish were tested.

A performance index above a certain level may be necessary for the survival even of fish with a normal performance rating. A very small fish may have the normal swimming ability for fish of this size but this ability may not be sufficient to allow him to escape predation. Survival studies with chinook salmon at the Spring Creek Hatchery in Washington (Junge and Phinney, 1963) indicate a much greater survival rate for fingerling releases than for releases of fry. Cope and Slater (1957) evaluated spring and fall releases of chinook salmon from the Coleman Hatchery in California and found a higher survival rate in the larger fish released in the fall. Although the numerous factors affecting survival are quite complex, the size of the fish and its swimming ability at the time of release are certainly important. Experiments now in progress will further determine the importance of both size and stamina on the numbers of returning adult salmon.

It is concluded that both methods of evaluation are desirable, the performance index to determine swimming ability and the performance rating to determine the relation of the sample to the expected performance of the animal.

SUMMARY

A stamina tunnel for comparison of physical capabilities of fingerling salmonids has been designed, developed, and evaluated. The design is such that water velocities varying from 0.4 feet per second up to 5.0 feet per second may be produced, increased, and reproduced in practically infinite velocity increments. The velocities are produced by means of an axial flow pump which creates head differentials between the intake and outlet of a 12-inch-diameter, 6-foot-long, plastic tube. The increments are controlled by regulation of the pump speed.

A testing pattern containing several velocity plateaus correlated with fixed exposure times has been developed for the testing of fingerling salmonids varying in weight from 1 to 50 grams.

A performance index has been evolved to assess the stamina of the individual fish samples and a performance rating to evaluate the significance of differences between samples.

The controllable variables affecting performance include fish size, water temperature, oxygen content, sampling technique, sample size, starvation period before testing, and recovery period before retesting. The existence of such variables makes the standardization of testing techniques imperative. Differences in performance exist also between species and races within species of salmon and trout.

By means of the tunnel it has been possible to demonstrate that varying methods of artificial propagation measurably affect the physical stamina of salmonids owing to conditions imposed by disease, nutrition, and environment.

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